



*Acta*

**OTO-LARYNGOLOGICA**

**VOL. 80 • JULY-AUGUST 1975 • No. 1-2**

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## AN EVOKED RESPONSE STUDY OF THE FIRST-ORDER DIFFERENCE TONE IN MAN

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(Received August 13, 1974)

**Abstract** The amplitude of the  $N_1-P_2$  component of a response evoked by a 250 Hz test stimulus presented once each 5 sec was measured. Interposed at a rate of one per sec were either 1) a 4 000 Hz tone, 2) a 4 000 Hz and a 250 Hz tone presented simultaneously which generated within the ear an audible 250 Hz difference tone, or 3) a 250 Hz tone. The results indicated that the  $N_1-P_2$  component when preceded by the 4 000 Hz plus 4 250 Hz stimulus pair was larger than when preceded by a 250 Hz tone, but smaller than when preceded by a 4 000 Hz tone. Apparently the processing of the difference tone is unlike that of a sinusoid of the same frequency or that of a high frequency sinusoid which participates in the generation of the difference tone. Parallel electrophysiological data from cochlear recordings are discussed.

When two tones, differing in frequency, are presented simultaneously at the higher intensity levels, a multitude of additional tones called combination tones, can be detected under suitable conditions of observations. One clearly audible combination tone is the first order difference tone whose tonal frequency corresponds to  $h-l$  where  $h$  and  $l$  are the frequencies of the higher and lower primary tones, respectively. The question which motivated the present experiment relates to the processing of the first order difference tone. Is it processed in the same manner as a sinusoid of the same frequency or as one member of the pair of primary tones which elicit the difference tone? We designed an evoked response experiment on humans as a

means toward answering this question. But first, a word about the human auditory evoked response as a suitable candidate for the task. The most prominent feature of the auditory evoked response is the  $N_1-P_2$  component, and its amplitude usually serves as the dependent variable when studying the influence of stimulus conditions on the electrical response of the brain. Yet when examining the form of the response, one cannot deduce the auditory stimulus which evoked it. For example, the response evoked by a first order difference tone will resemble that evoked by any other sound. There is however, a way to get a handle on the problem. Specifically  $N_1-P_2$  amplitude to a sinusoid of one frequency will suffer a greater decrement if preceded by a stimulus of identical frequency than if preceded by a stimulus of some other frequency (Butler, 1968). In our experiment we simply preceded a 250 Hz test stimulus by either 1) another sinusoid of 250 Hz, or 2) a pair of primary tones (4 000 and 4 250 Hz) which generated a first order difference tone of 250 Hz, or 3) a 4 000 Hz tone which, of course, was one member of the tonal complex that elicited the difference tone. We reasoned that if both the 250 Hz sinusoid and the difference tone have the same suppressive effect on the test stimulus, one could argue that they are processed in a similar manner. If on the other hand, the influence of the difference tone mimicked that of the 4 000 Hz tone, then the inference would be that these two sets of stimuli are processed similarly.

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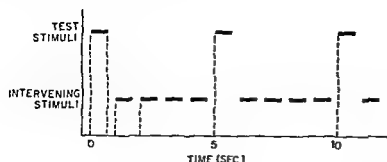


Fig 1 The temporal sequence of stimulus presentation. The test stimulus was at 250 Hz.

## MAIN EXPERIMENT

### Method

Fig 1 illustrates the sequence of stimulation. The duration of all stimuli was 100 msec, their rise-fall time was 10 msec. The test stimulus was presented once each 5 sec, the intervening stimuli were delivered once each sec between successive test stimuli. Four conditions of intervening stimulation were established. For Conds 1, 2, and 3, 4 000 Hz, 4 000 plus 4 250 Hz, and 250 Hz intervened, respectively. In Cond 4, no auditory stimuli were interposed. To preclude the possibility of the difference tone (Cond 2) being produced by the stimulus generating equipment and hence contained in the auditory input to the ear, the 4 000 Hz tone was generated and controlled by one audio channel and the 4 250 Hz was generated and controlled by a separate

Furthermore, each tone activated a different loudspeaker. In fact, 3 loudspeakers were utilized—1 for the 4 000 Hz stimulus, 1 for the 4 250 Hz stimulus which was also used as the source of the 250 Hz intervening stimulus when appropriate (Cond 3), and the 3rd loudspeaker generated the 250 Hz test stimulus. The loudspeakers, 4-inch diam, were housed in a  $6\frac{1}{2} \times 6\frac{1}{2} \times 6$  inch wooden cabinets. These cabinets were bound together in a pyramidal pattern and fixed to a vertical post. The listener was seated in a chair equipped with a headrest. The chair was situated so that the listener's left ear corresponded in height to the center of the pyramid of loudspeakers. Distance between the pyramid's center point and the listener's pinna was approximately 8 inch.

Stimulus intensity for the 4 000 and 4 250 Hz primary tones was 80 dB SPL as measured at a position which, during testing, would have been

occupied by the listener's left pinna. At this intensity, the loudness of the 250 Hz difference tone was estimated by introducing an exploration tone of 253 Hz. The intensity of the exploration tone which produced the best beats as agreed upon by 3 trained listeners was 45 dB SPL. Hence, the intensity of the 250 Hz test stimulus was set at 45 dB SPL as was that of the 250 Hz intervening stimulus.

Referring again to Fig 1, an experimental condition consisted of a sequence of test stimuli intervening stimuli which continued until the test stimulus had been presented 75 times. On response evoked by the test stimuli were transmitted to the computer (FabriTek, Model 107). These were recorded between the vertex and paired earlobes. A head strap secured the vertex electrode, plastic clips secured the earlobe electrodes. The waveform of the summed response was displayed on an oscilloscope screen and the amplitude of the  $N_1-P_2$  component was calculated (see Butler, 1972).

Eight persons, whose hearing acuity was within 15 dB of audiometric zero (ISO Standard: 1964) for the frequencies 250–8 000 Hz, served as subjects. A subject was given 4 test sessions and each session consisted of the 4 experimental conditions. A  $4 \times 4$  Latin square governed the order in which the experimental conditions were presented. Rows represented subjects, columns represented ordinal position of tests within a session, and Latin letters represented the experimental conditions. For each listener, the  $N_1-P_2$  component for the various experimental conditions was summed over the 4 test sessions. These values, 1 for each experimental condition, were then ordered for further statistical treatment.

Before describing our results, it should be

mentioned that the frequency of the test and intervening stimuli were monitored repeatedly during the course of a session by means of a Hewlett-Packard Electronic Counter (Model 5221A). We immediately corrected for any slight drift of the oscillators. Testing was conducted in a sound treated room.

## Results

Fig. 2 summarizes the data.  $N_1$ - $P_2$  amplitude recorded under Cond. 4 was taken as the normal value of a response to the 250 Hz test stimulus since no other auditory stimuli intervened to affect the amplitude adversely. It is evident from this histogram that when a 250 Hz tone intervened (Cond. 3), response amplitude to the test stimulus was reduced severely. The 2 primaries which produced a 250 Hz difference tone failed to exert a comparable suppressive effect (Cond. 2). Yet, they had a greater suppressive effect than did the presentation of only one of the primaries (Cond. 1). Mean amplitudes of the  $N_1$ - $P_2$  component in  $\mu$ V were 4.5, 3.4, and 2.0 for Conds. 1 through 3, respectively. With no auditory stimuli intervening, mean response amplitude to the test stimulus was 5.6  $\mu$ V (Cond. 4). Not only were differences in response amplitudes among experimental conditions statistically significant ( $p < 0.01$ ) as indicated by a variance analysis, but each mean amplitude value listed above differed significantly from all the other mean values ( $p < 0.01$  "F" statistic). In brief, numerical differences between mean response amplitudes associated with the 4 experimental conditions were small, but highly consistent. Each listener's data showed the same pattern as that which characterized the group data.

## Comment

Upon further reflection there appeared to be a problem inherent in the design of the experiment. Specifically, how can one expect high frequency stimuli (Cond. 2) to exert the same influence on the response to the test stimulus as exerted by a low frequency stimulus (Cond. 3)? Their physical characteristics differ so widely. And whereas the high frequency primaries gener-

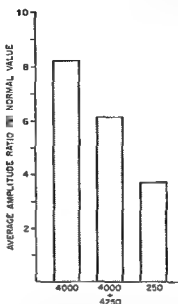


Fig. 2 The relative amplitude of the response to the test stimulus as a function of the different intervening stimuli whose frequencies are expressed in Hz.

ated a low frequency difference tone, their mere presence may have in some way lessened the effect on the response to the low frequency test stimulus. There is no satisfactory solution to this dilemma, but we conducted additional tests, the outcome of which seemed to have attenuated the seriousness of the problem.

## SUB-EXPERIMENT 1

### Method

Again we investigated the influence of intervening stimuli on the response amplitude evoked by the test stimulus. In Part 1, our intervening stimuli were 1) 4000 plus 4250 Hz presented simultaneously, and 2) 4000 plus 250 Hz presented simultaneously. The test stimulus remained at 250 Hz. Intensity, duration, and rise-fall time for the various frequencies were the same as those described for the main experiment. The same listeners participated and each was given 2 test sessions. A session was comprised of 2 presentations of each intervention stimulus condition in an ABBA order. "A" represents the 4000-4250 Hz stimulus and "B" represents the 4000-250 Hz stimulus.

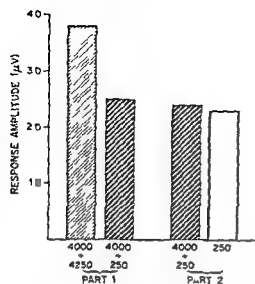


Fig 3 Response amplitude to the test stimulus under conditions of different intervening stimulation

pairing. Responses evoked by 75 test stimuli were summed for each condition of intervening stimuli. Part 2 was conducted identically except the intervening stimuli were 1) 4 000 plus 250 Hz presented simultaneously, and 2) 250 Hz presented alone.

### Results

regard to Part 1, the stimulus pair which produced the difference tone has a significantly less effect ( $p < 0.01$ ) on the responsiveness to the 250 Hz test stimulus than did the stimulus pair, 4 000 plus 250 Hz, as indicated by the Wilcoxon Matched Pairs Signed-Ranks test. When applying the Wilcoxon test to the Part 2 data, no differences were found between the 4 000 plus 250 Hz intervening stimulus pair and the intervening 250 Hz tone with respect to suppressing the response to the 250 test stimulus. Each had about the same effect. A histogram based on mean response amplitudes to the test stimulus associated with those intervening stimuli used in Parts 1 and 2 is shown in Fig 3.

### Comment

To produce a maximal suppressive effect on the response to a 250 Hz test stimulus it apparently does not matter whether the 250 Hz intervening stimulus is paired with a high frequency tone, in

this case, 4 000 Hz, or whether it is presented alone. The manner by which the 250 Hz intervening tone is generated, however, is critical. It must be contained in the auditory input for the suppressive effect to be significantly reduced. If 250 Hz intervening "experience" is a consequence of nonlinear distortion within the ear

## SUB EXPERIMENT 2

### Method

Another possible explanation for the finding that the intervening difference tone affected the response to the test stimulus less than did the 250 Hz sinusoid is that the loudness of the difference tone may have been lower than that of the 250 Hz tone. Butler (1968) reported that an intervening stimulus which is softer than the test stimulus has a lesser suppressive effect than does an intervening stimulus whose loudness is equal to that of the test stimulus. And it should be pointed out that the method of best beats which we used to set the intensity of the 250 Hz test stimulus as well as the 250 Hz intervening stimulus lacks precision. There is a range of intensity values at which the beats appear equally loud or nearly so. To assuage our doubts we ran additional tests where the loudness of the difference tone was deliberately set above that of the 250 Hz sinusoid. Specifically, the 250 Hz test stimulus and the 250 Hz intervening stimulus were reduced by 15 dB. Since we obviously wanted to avoid distortion in our stimuli system, we only boosted the 4 000 and 4 250 Hz primaries by 2 dB. Under these conditions, the difference tone was judged to be unquestionably louder than the 250 Hz sinusoid. With the same 8 persons, we measured their response amplitudes to the 250 Hz test stimulus when the 250 Hz sinusoid and when the 250 Hz difference tone served as the intervening stimuli. Again 21 sessions were given with each subject being tested on each experimental condition a total of four times.

### Results

Notwithstanding a clear difference in loudness between intervening stimuli, the results were the

ame as those found in the main experiment. All showed greater response amplitudes to the test stimulus when the stimuli producing the 50 Hz difference tone intervened between successive test stimuli than when the 250 Hz sinusoid intervened. Mean response amplitude for the former was  $2.9 \mu\text{V}$ , that for the latter was  $1.8 \mu\text{V}$ .

## DISCUSSION

Our main effect was unequivocal. The stimulus producing the difference tone affected the response to the test stimulus less than did a sinusoid of the same frequency (250 Hz), but more than did one of the primaries (4 000 Hz). The magnitude of these differences exceeded the 1 percent level of confidence. We infer then that the difference tone was processed differently than either 1) the primaries or 2) a sinusoid whose frequency was identical to that of the difference tone. The data from additional tests rule out some possible explanations. It was not the presence *per se* of a high frequency tone which attenuated the suppressive effect of the difference tone on the test stimulus. If a 250 Hz sinusoid is present in the original stimulus, it will severely reduce the response amplitude to a 250 Hz test stimulus. It matters not whether the stimulus complex also contains a high frequency tone (Sub-experiment 1). Nor was it that the difference tone may have been softer than the test stimulus, make it appreciably louder and it still does not affect the responsiveness to the test stimulus as much as a softer sinusoid identical in frequency (Sub-experiment 2).

Are there other data which suggest that a first-order difference tone is treated by the auditory periphery as neither one of the primaries which elicits the difference tone, nor as a sinusoid of the same frequency? There are, but they are generated by vastly different experimental procedures, viz., electro-physiological recordings of cochlear microphonics (CM) in the guinea pig cochlea. DeBoer & Six (1960) published the original findings. Dallos and his associates (Sweetman & Dallos, 1969; Worthington & Dallos, 1971) confirmed these findings and went on to

develop a theoretical structure for them. Their account runs as follows. At relatively low intensity levels of the primaries, the CM corresponding to the difference tone reaches its maximum amplitude somewhat apically to the place where the higher primary CM is maximal. At relatively high intensity levels of the primaries, the difference tone CM is distributed along the basilar membrane similarly to that of a sinusoid identical in frequency. A two-stage process of cochlear distortion is proposed. Stage 1, operating at the lower intensity levels, reflects an electromechanical (hair cell transducing process) distortion. Stage 2, operating at the higher intensity levels, reflects a hydromechanical distortion. The latter process initiates its own traveling wave which then behaves as any other traveling wave initiated by a sinusoid of corresponding frequency. Our data placed in this Stage 1 - Stage 2 context, would stand at the transition point. They reflect neither stage unequivocally and the reason for this may be that the intensity of our primaries (80 dB SPL) falls in the middle of the range explored by Worthington & Dallos (50 to 115 dB SPL).

Worthington & Dallos, taking cognizance of their finding that the first-order difference tone CM can have its maximum at a place along the basilar membrane different from that of a CM elicited by a sinusoid of corresponding frequency, asked the question: Is this difference tone audible? If we are at all justified in relating our data to theirs, the answer is "yes". Our difference tone was clearly audible and it obviously was not being processed in the same way as a sinusoid of corresponding frequency.

## ZUSAMMENFASSUNG

Die Amplitude der  $N_1$ - $P_2$ -Komponente des evozierten Potentials für einen 250-Hz Ton gemessen zwischen dem Vertex und den beiden Ohrklappchen, wurde in drei experimentellen Situationen untersucht. Der Testton wurde alle 5 Sekunden einmal dargeboten. Dazwischen wurden — einmal pro Sekunde — entweder 1) ein 4 000-Hz Ton, 2) gleichzeitig ein 4 000-Hz Ton und ein 4 250-Hz Ton was zu einem hörbaren 250-Hz Differenzton führte oder 3) ein 250-Hz Ton interpoliert. Wir wussten von früheren Untersuchungen, dass der 250-Hz Ton die

Amplitude des evozierten Potentials für den 250-Hz-Testton stärker reduziert wurde als der 4 000-Hz-Ton. Die Frage war, ob das Tonpaar das den 250-Hz-Differenzton veranlasste, in seinem Effekt dem 250-Hz-Ton oder dem 4 000-Hz-Ton gleichen wurde. Die Resultate zeigten, dass die Amplitude der  $N_1$ - $P_2$ -Komponente nach dem 4 000/4 250-Hz-Tonpaar grösser war als nach dem 250-Hz-Ton, aber kleiner als nach dem 4 000-Hz-Ton. Wir folgerten, dass der Differenzton anders verarbeitet wird als ein Sinuston derselben Frequenz, und auch anders als ein Sinuston mit hoher Frequenz, wie er bei der Produktion des Differenztons mitwirkte. In der Diskussion nahmen wir Bezug auf elektrophysiologische Studien der Cochlea, die offensichtlich vergleichbare Resultate lieferten.

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## CORTICAL EVOKED POTENTIALS RECORDED FROM THE GUINEA PIG WITHOUT AVERAGING

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**Abstract** Potentials evoked by tonal pulses and recorded with a monopolar electrode on the pial surface over the auditory cortex of the guinea pig are presented. These potentials are compared with averaged potentials recorded in previous studies with an electrode on the dura. The potentials recorded by these two techniques have similar waveforms, peak latencies and thresholds. They appear to be generated within the same region of the cerebral cortex. As can be expected, the amplitude of the evoked potentials recorded from the pial surface is larger than that recorded from the dura. Consequently averaging is not needed to extract the evoked potential once the dura is removed. The thresholds for the evoked cortical potential are similar to behavioral thresholds for the guinea pig at high frequencies; however, evoked potential thresholds are elevated over behavioral thresholds at low frequencies. The removal of the dura and the direct recording of the evoked potential appears most appropriate for acute experiments. The recording of an evoked potential with dura electrodes employing averaging procedures appears most appropriate for chronic studies.

Several types of bioelectric potentials have been recorded from the human scalp (Davis, 1965, Cody & Buckford, 1965, Cody & Kloss, 1968, Rapin & Bergman, 1969). These potentials have been especially useful with infants, the mentally retarded and malingerers. Similar potentials have been recorded from experimental animals in an attempt to quickly assess their auditory acuity.

In order to test the auditory ability of animals thresholds for the evoked cortical potential have

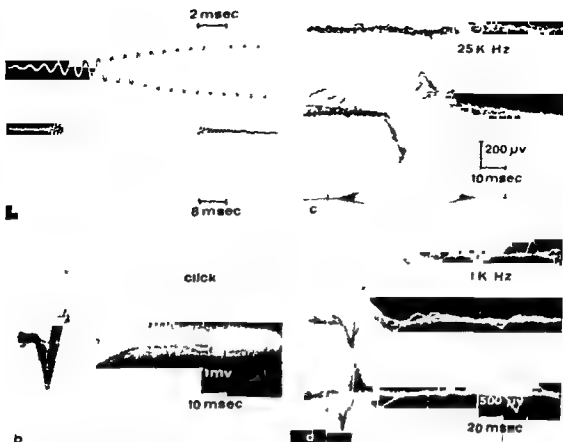
The data presented in this article were collected in partial fulfillment of the requirements for the doctoral degree in the Department of Medical Psychology, University of Oregon Medical School. The work was in part sponsored by Program Project no. NS 09889.

been obtained for the cat (Hind & Schuknecht, 1954, Kimura et al., 1956, Hattori et al., 1971) as well as for the guinea pig. In the guinea pig, Djahjian & Cody (1973) and Hattori (1970) have used averaging techniques to obtain evoked potentials from dura electrodes. In one study (Kern et al., 1969) the vertex response was recorded from the guinea pig. In a number of recent studies (Walloch et al., 1971a, b, 1973a, b, 1974a, b, Cowden & Walloch, 1973) I have removed the dura and placed the recording electrode directly on the pial surface. In this manner, the evoked cortical potential could be recorded without averaging. The present paper compares and contrasts the records obtained from electrodes on the pia without averaging with those obtained from electrodes on the dura with averaging. The data contained in the present paper have not been reported except as a brief communication (Walloch, 1971a).

### METHOD

Eleven guinea pigs of the Topeka stock weighing 400-750 g were used in this study. Each guinea pig had an active Preyer reflex. The guinea pig was deeply anesthetized with diallylbarbituric acid (60 mg/kg) and urethane (240 mg/kg). In other studies (Walloch et al., 1974a, b) sodium pentobarbital (45 mg/kg) was used with equal success. Rarely, a supplemental dose was needed to maintain anesthesia. A tracheotomy was per-





**Fig. 1** Record of the stimulus and evoked responses. Negative is up. (a) Averaged output of the calibrated microphone to a standard tonal pulse, showing that the sound pulse was free of significant onset transients. (b) Superimposed cortical evoked responses to repeated clicks. (c) Cortical responses evoked by 25 kHz tonal pulses. Top tracing shows lack of evoked activity following repeated stimulus presentations at 21 dB SPL. The

middle tracing shows a clear evoked response following repeated stimulation at 31 dB. The bottom line indicates the duration of the tonal pulse. (d) Cortical responses to 1 kHz tonal pulses. The top line shows absence of cortical activity following repeated stimulation at 25 dB. The middle and bottom tracings show a well-developed response at 35 and 45 dB respectively.

formed and the animal was mechanically ventilated.

In the present experiment the guinea pig's rectal temperature was continuously monitored (Yellow Springs Instrument 46 TUC) and maintained at 38°C. When the body temperature falls below 35°C, the evoked cortical potential disappears. The evoked potential returns upon warming the animal. Above 40°C, the evoked potential deteriorates and in this case the deterioration is not reversible.

Both pinnae were amputated to facilitate placement of the animal in a stereotaxic unit (David Kopf). The scalp and skull flap were removed to expose the entire left hemisphere.

To remove the dura, an old tungsten micro-

electrode was used, though any sharp thin probe would suffice. The dura was impaled medially from the auditory area and lifted away from the cortex. An incision was made beneath the microelectrode with Vannas scissors. This incision was expanded anterolaterally. The dura was reflected anteriorly. Some bleeding occurred on occasion while the dura was being removed. When this occurred, it was best to proceed quickly with the reflection of the dura. With the dura reflected, the bleeding could then be controlled with the use of gelfoam. Most often the bleeding came from the soft tissue surrounding the cortical exposure. Only rarely did the bleeding stem from the cortex itself.

After the dura was reflected, warm mineral oil

was allowed to flow across the exposed cortex. However, the mineral oil was not found to be needed in subsequent studies. A silver ball electrode (0.18 mm) was moved across the temporal area of the guinea pig in 1 mm steps. The impedance of the electrode was about 2 kilohms. At each cortical position studied, the threshold for an auditory evoked potential was obtained at 18 frequencies ranging from 0.1 to 40 kHz.

In order to obtain these evoked potentials, sound was delivered through the hollow ear bar contralateral to the exposed cortex. A General Radio wave analyzer (1900A), Monsanto digital counter (103A), Simpson voltmeter, Grason-Stadler electronic switch (892E), Grason-Stadler interval timer (4711), General Radio decade attenuator (1450), McIntosh power amplifier (M 210-B) and a specially designed power attenuator shaped the electrical signal which drove the Western Electric 555 speaker. The speaker was connected to the hollow ear bar as a closed sound system. The rate of stimulation was once every 2 seconds. Each tonal pulse was 50 msec in duration and had a rise time of 10 msec. At the end of each day's work, a 1/4 inch Bruel & Kjaer calibrated microphone was substituted for the animal and the sound at the end of the hollow ear bar was thus measured (Fig. 1a).

The evoked cortical potentials were initially amplified using a Keithley differential amplifier (103). The filters were set at 100 and 3000 Hz. The calibration of this amplifier has been previously described (Meikle & Copeland 1973). After the initial amplification, the potentials were displayed on a Tektronix storage oscilloscope (564B).

## RESULTS

Acoustically evoked potentials were recorded from a restricted area within the temporal region of the cerebral cortex. Other investigators have identified this region as the auditory cortex (Ödkvist et al. 1973; Ziegler 1964; Kayser & Legoux 1963). The first wave was always surface positive and it had a peak latency of 10–15 msec. A surface negative wave with a peak

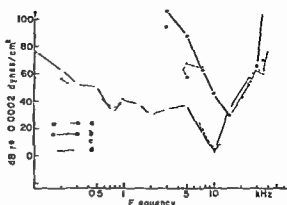


Fig. 2. Threshold curves for the evoked responses recorded from four cortical positions in a single guinea pig. All four positions were in the temporal region and within a few millimeters of each other. See text for a fuller discussion of these data.

latency of 20–40 msec generally followed the initial positive wave. A second surface positive wave was observed rarely and only at very high sound intensities. No later waves were observed.

In Fig. 1b, an evoked potential to a click is shown. Clicks were used in a few pilot animals to determine the location of the auditory cortex. The waveform of the click evoked potential is highly similar to that evoked by tonal pulses. No attempt was made to obtain the threshold of these click evoked potentials; however, they were very useful in determining the latencies of the evoked potentials.

In Fig. 1c, the cortical responses to a 25 kHz tone are shown. The top tracing shows a lack of an evoked response to repeated stimulation at 21 dB re 0.0002 dynes/cm². The middle tracing shows a 650  $\mu$ V (p-p) response to repeated stimulation at 31 dB.

In Fig. 1d, the cortical responses to a 1 kHz tone are shown. In the top tracing, no evoked activity is apparent following repeated stimulation at 23 dB. In the middle trace, a 1 mV potential was evoked by repeated stimulation at 33 dB. These thresholds were stable over 5 and 8 hour recording sessions.

In Fig. 2, the thresholds for the evoked potential recorded from four positions on the auditory cortex of a single guinea pig are shown. All

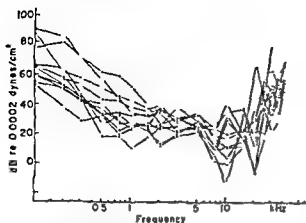


Fig 3 Thresholds for the evoked response from 11 guinea pigs. The entire left hemisphere of each guinea pig was explored for evoked cortical activity. At each frequency, the threshold represents the lowest intensity tonal pulse that evoked cortical activity regardless of the position of the recording electrode.

four positions were within 4 mm of each other. Two of the positions (*a* and *b*) were sensitive to high frequency tones, and tonal pulses below 1.5 kHz were totally ineffective in evoking a potential. One position (*c*) was sensitive to low frequency stimuli. One position (*d*) was sensitive to

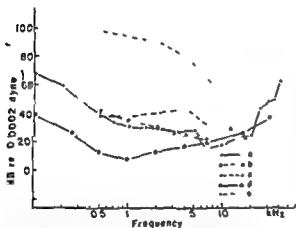


Fig 4 Comparison of the mean of the threshold curves plotted in Fig 3 with other measures of auditory acuity in the guinea pig. (a) Mean of the threshold curves obtained in the present study. (b) Thresholds for the averaged evoked potentials reported by Hattori (1970) from the dura overlying the auditory cortex of the guinea pig. (c) Thresholds for the averaged evoked potentials recorded by Djalilian (1973) from the dura overlying the auditory cortex of the guinea pig. (d) Behavioral threshold obtained by Miller (1966) in the guinea pig. (e) Vertex potential threshold obtained by Kern (1969) from dura electrodes in the guinea pig.

both low and high frequency stimuli. Consequently, in order to obtain the lowest threshold for the evoked cortical potential at all frequencies without averaging, a number of cortical positions must be sampled.

In Fig 3, the thresholds for the auditory evoked potential obtained from 11 guinea pigs are shown. The entire left hemisphere was sampled in 1 mm steps. At each frequency, the lowest sound intensity that could evoke a cortical potential regardless of the recording position is shown. Consequently, each threshold curve represents potentials recorded from a number of cortical positions.

Fig 4 compares the mean of the threshold curves shown in Fig 3 with other comparative measures of the auditory acuity in guinea pigs. The thresholds obtained by computing averaged evoked potentials recorded from the dura overlying the auditory cortex of the guinea pig (Djalilian & Cody, 1973; Hattori & Shoyama, 1970) are very similar to those obtained in the present study. These evoked potential thresholds were similar to the behavioral threshold of guinea pigs (Miller & Munson, 1966) at high frequencies, but these evoked potential thresholds were elevated over behavioral thresholds at the low frequencies.

## COMMENT

The thresholds reported in the present study are very similar to those reported by Hattori & Shoyama (1970) and to those reported by Djalilian & Cody (1973) (Fig 4). Hattori & Djalilian used an averaging computer to record evoked potentials from electrodes on the dura over the auditory cortex. The potentials recorded in all three studies had, 1) similar waveforms, 2) latencies, 3) thresholds, and 4) spatial distributions on the cerebral hemisphere. Consequently the potentials recorded in the three studies appear to have the same origin.

For certain acute experimental problems, the removal of the dura and the direct recording of the evoked cortical potential appears more appropriate. The large amplitude of the evoked potential (Fig 1*c, b*) at threshold makes an

aging unnecessary. Consequently, thresholds can be obtained much more rapidly with direct recording techniques than with averaging procedures. While 50 stimulus presentations were used to obtain an averaged evoked potential in Djalian's (1973) and Hattori's (1970) studies, only 2 stimulus presentations were needed in the present study.

On the other hand, for certain chronic experimental problems, the recording of averaged evoked potentials from an electrode on the dura appears most appropriate. The opening in the skull can be easily closed and the guinea pig may be allowed to recover from the anesthesia.

The choice of the position on the cerebral cortex from which to record the evoked potentials greatly influences the thresholds obtained. The position used by either Hattori (1970) or Djalian (1973) appear appropriate for chronic studies. For acute studies in which the dura has been removed, the cortex can be quickly searched for an appropriate position. At each cortical position, the threshold for a potential evoked by 1 kHz and 10 kHz tonal pulses can be quickly ascertained. If the 1 kHz threshold is below 45 dB and the 10 kHz threshold is below 35 dB, the cortical position is suitable for the experiment. From such a position a complete threshold curve would appear similar to Fig. 2d.

The behavioral threshold for pure tone stimuli have been obtained several times for the guinea pig (Horton, 1933, Anderson & Wedenberg, 1965, Miller & Murray, 1966, Heffner et al., 1971). The thresholds depicted in Fig. 4d are representative of other behavioral determinations. The thresholds for the evoked potentials obtained in the present study are similar to the behavioral thresholds at the high frequencies. The evoked potential thresholds are elevated over the behavioral thresholds at the low frequencies. The majority of the positions on the auditory cortex are most sensitive to high frequency stimuli. While 63% of the positions had best frequencies over 5 kHz, only 37% had best frequencies below 3 kHz (Wallock, 1971c). Perhaps this can explain the relative insensitivity of the evoked potential to low frequency stimuli.

On the other hand, differences in 1) sound measurement procedures, 2) the rise time, and 3) duration of the tones cannot be discounted as contributing to the differences in the evoked potential and the behavioral thresholds.

The vertex response of the guinea pig (Kern et al., 1969) is highly elevated over the other measures of auditory acuity shown in Fig. 4. This contrasts with the human vertex potential which is similar to the thresholds obtained by conventional pure tone audiometry (Cody & Bickford, 1965, Cody & Klass, 1968, Rapin & Bergman, 1969). The reason for this difference between the guinea pig and human vertex potential is not apparent.

The evoked potential from the auditory cortex has been used to study the effects upon the auditory system of intense sound stimulation (Hattori, 1970, 1971) ethacrynic acid (Wallock, 1974a) and mechanical lesions of the cochlea (Kimura et al., 1956, Hind & Schuknecht, 1954). The evoked potential has also been used to investigate various placements of electrodes for the electrical stimulation of the ear (Wallock et al., 1973a, 1974b). In the future, we can expect that the evoked potential will be used to study an ever-increasing scope of problems.

## ZUSAMMENFASSUNG

Aktionspotentiale der Hörinde wurden bei Meerschweinchen durch Tonimpulse hervorgerufen und mittels monopolarer Elektrode an der pia mater abgeleitet. Sie wurden mit Durchschnittsaktionspotentialen verglichen, die in früheren Untersuchungen von der dura mater abgeleitet worden waren. Diese mit verschiedener Methodik erhaltenen Potentiale ähneln einander hinsichtlich Verlaufsform, maximaler Latenzzeit und Schwellenwert. Sie scheinen in derselben Gegend der Hirnrinde zu entstehen. Wie zu erwarten ist die Amplitude der von der pia mater abgeleiteten Aktionspotentiale grösser als die von der dura mater. Daher ist es nicht notwendig Aktionspotentiale zu ermitteln, wenn einmal die dura mater beseitigt ist. Hervorgerufene Rindenpotentiale haben beim Meerschweinchen bei hoher Reizfrequenz ähnliche Schwellenwerte wie Verhaltensreaktionen. Jedoch liegen bei niedrigen Frequenzen die Schwellenwerte von hervorgerufenen Potentialen höher als die Schwellenwerte von Verhaltensreaktionen. Beseitigung der dura mater und direktes Registrieren des Aktionspotentials scheinen am besten für akute Experimente geeignet zu sein, während

Registrieren von Durchschnittsaktionspotentialen mit dura Elektroden sich am besten für chronische Experimente zu eignen scheint

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## BIASING OF THE SUMMATING POTENTIALS

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*(Received October 9, 1974)*

**Abstract:** Summating potentials (SP) were recorded in the guinea pig cochlea, utilizing the differential electrode technique. The effects of biasing the cochlear partition on the SP were observed under three different conditions: 1. electrical biasing—direct current polarization; 2. stimulus biasing—mechanical bias induced by low frequency sound stimulation; 3. COCB biasing—electrical stimulation of the crossed olivo-cochlear bundle. All three forms of biasing were found to have similar effects on the SP which can be described in terms of the absolute direction of change in the potential. Under negative current (scala vestibuli negative, scala tympani positive), the negative phase of the stimulus bias (deflection of the cochlear partition towards scala vestibuli) or stimulation of the COCB, the SP is made more positive or less negative, as the case may be. In other words, the negative component of the differentially recorded SP is depressed whereas the positive component is enhanced. The dominant influence of all three forms of biasing appears to be on mechano-electric distortion arising in the hair-cell transduction process.

The summating potential (SP) is by definition a distortion product. It is typically observed as a d.c. response to a sinusoidal (a.c.) input, namely a pure tone sound stimulus (Dallos, 1973). Over the past several years a series of experiments have been carried out in this laboratory which were designed to analyse the relative contribution of mechanical and electrical nonlinearities to the generation of various compo-

nents of the SP in the cochlea (Durrant & Dallos, 1972, 1974). The findings of these studies have at least contributed to the support of a unified concept of distortion production in the cochlea. That is to say, the SP may be attributed largely to the same basic nonlinearity(ies) underlying such phenomena as harmonic distortion, intermodulation distortion, and interference observed electrophysiologically (Engelbreton & Eldredge, 1968). It has also been suggested that the underlying distortion process(es) is strongly electromechanical in origin. In other words, the distortion which is manifested in the electrophysiological responses of the cochlea appears to arise predominantly from a nonlinear (hair-cell) transducer process rather than a purely hydromechanical process such as nonlinear wave motion which leads to asymmetrical displacement of the basilar membrane and the formation of Bekesy's eddies (Tonndorf, 1970). That such hydromechanical nonlinearities may exist is not the point of argument, but rather it is asserted that components such as the SP arise at a later stage of cochlear function, namely at the site of the transducer.

Electrical and mechanical forms of biasing of the cochlear partition can be observed to have similar effects on the SP, as suggested by the findings of two earlier studies—one by Butler & Honrubia (1963) and the other by Davis et al (1958). Data from these two studies are shown in Fig 1a and b, respectively. The data have been replotted to show normalized response magnitude as a function of the independent variable. The data presented in panel b demon-

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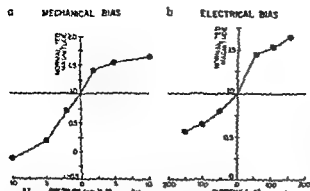


Fig 1 Effects of mechanical and electrical forms of biasing of the cochlear partition on the negative summing potential. The graphs shown are adaptations of plots presented by (a) Butler and Honrubia (1963, composite of figures 1 and 2) and (b) Davis et al (1958 figure 7). (a) Normalized response magnitudes (see text) are plotted as a function of hydrostatic pressure selectively applied to scala tympani (ST) and scala vestibuli (SV). (b) Normalized response magnitude as a function of current level. Positive (+) current, SV positive and ST-negative. Negative (-) current, ST positive and SV-negative.

strate the effects on the negative SP of selectively increasing the intracochlear hydrostatic pressure in either scala tympani or scala vestibuli. The effects of applying direct current across the cochlear partition are shown in panel b. (The normalized magnitude is obtained by simply dividing the magnitude of the responses observed under the experimental condition by the magnitude under the control condition, that is under zero pressure or current.) The SP may also be altered by electrically stimulating the crossed olivo-cochlear bundle (Konishi & Slepian (1971a, b) have described these effects, but their data cannot be readily compared with those shown in Fig 1, since a direct analog of the "bias level" functions illustrated in this figure does not exist for the COCB biasing technique. Current level alone does not determine the effectiveness of COCB stimulation which depends upon the use of pulsed current and the parameters of the pulses (level, duration, and frequency). Nevertheless, the fact that the SP can be changed under the influence of these different forms of biasing of the cochlear partition is impressive and suggests the basis of a useful comparative analysis. This may lead to a more exacting means of

determining the dominant form of nonlinear contribution to the production of the SP.

The studies discussed above leave several questions open. First, is the apparent comparability of electrical and mechanical biasing illustrated by Fig 1, more than coincidence? Secondly, is it equally applicable to both positive and negative components of the SP? Third, what are the effects of COCB stimulation on the SP in any way analogous to the effects of electrical and mechanical biasing? In order to resolve these issues, it was necessary to examine the effects of all three forms of biasing on the SP under a variety of stimulus and recording conditions. The details of these investigations have been described elsewhere (Durrant & Dallos 1972, 1974, Gans, 1974). The purpose of

## METHODS

Guinea pigs were utilized throughout the studies. The animals were prepared with differential recording electrodes, generally in the first cochlear turn. The tone burst elicited responses were averaged by means of a digital computer. Since the probe stimuli were 1 phase coherent tone bursts, the microphonic responses elicited by the probe were cancelled through averaging. The sound stimuli were presented and monitored directly in a closed acoustic system.

Three experimental techniques were utilized to alter the SP, the details of which have been described elsewhere (Durrant & Dallos, 1972, 1974, Gans, 1974). Only a brief description is provided here.

### 1 D C polarization

Fluid filled pipettes were placed in the first cochlear turn of the guinea pig, one in scala vestibuli and the other in scala tympani, near the site of recording. Current was passed through these electrodes (typically 50  $\mu$ A) utilizing

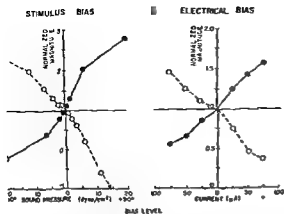


Fig. 2. Effects of different levels of bias on the positive (a) and negative (b) components of the DIF SP. (a) DIF<sup>+</sup> was elicited by a 10 kHz tone burst presented at 1 dB SPL, DIF<sup>+</sup>, 1 kHz at 90 dB. The bias stimulus was 70 Hz in the case of DIF<sup>-</sup> and 35 Hz for DIF<sup>+</sup>. The abscissa shows the RMS sound pressure of the stimulus. (b) DIF<sup>-</sup> was elicited by a 10 kHz tone burst and DIF<sup>+</sup> by a 200 Hz stimulus, both presented at 100 dB SPL.

instant current source. The SP responses were elicited with a 20 msec tone burst which will be referred to as the probe stimulus.

### Stimulus biasing

Under this procedure only recording electrodes were utilized. The probe stimulus was a brief tone pip which was presented in the presence of a steady tone of much lower frequency. The frequency of the probe stimulus was always more than 10 times that of the bias stimulus. The tone pip (probe) was usually 2 or 5 msec long with a rise-decay time equal to about one half its duration. The brief transient-like SP evoked by this stimulus exhibited characteristics not unlike that elicited by longer tone bursts with rectangular envelopes. The occurrence of the probe could be synchronized with a set phase of the low frequency bias stimulus. Since the basilar membrane vibrates in phase along most of its length at low frequencies (Bekesy, 1960), the microphonic evoked by the bias itself served as a means of selecting the appropriate phase of the biasing deflection. Usually, the probe was aligned with either the plus or minus 90° points of the relative CM phase.

### 3 COCB stimulation

Following surgical preparation to reveal the dorsal aspect of the brainstem, a pair of wire electrodes were placed in the floor of the fourth ventricle between the facial genua. Pulse trains fed through a photo-electric isolation unit were utilized to stimulate the COCB. The acoustic probe stimulus (tone burst with rectangular envelope as in the d.c. polarization studies) was presented 10 msec following the end of the shock or bias stimulus.

## RESULTS

The results of the electrical and stimulus biasing experiments may be described in terms of bias-level functions similar to those shown in Fig. 1, as seen in Fig. 2. Data are presented for both the DIF<sup>-</sup> and DIF<sup>+</sup> components. Results from the stimulus biasing studies are shown in panel a in which case the DIF<sup>-</sup> was elicited by a 10 kHz tone pip presented at 90 dB SPL (sound pressure level re 0.0002 dyne/cm<sup>2</sup>), and DIF<sup>+</sup> by 1 kHz at 90 dB. It can be seen that the magnitude of the DIF component is enhanced (i.e. normalized magnitude > 1) under the -90° biasing condition, but it is depressed (normalized magnitude < 1) under the +90° condition. The opposite is true for the positive component. A comparable set of data from the polarization studies is presented in panel b. In this instance DIF<sup>-</sup> was elicited by a 10 kHz tone burst and DIF<sup>+</sup> by 200 Hz, both presented at 100 dB SPL. When scala vestibuli is made more positive with respect to scala tympani (+ current), DIF<sup>-</sup> is enhanced, and DIF<sup>+</sup> depressed. The opposite is true of negative currents. Thus, the effects of stimulus and electrical biasing upon the negative SP component are quite similar to those evidenced in Fig. 1, whereas the reverse of these effects are seen in the case of the positive component.

The apparent differential effects of biasing on the two components of the DIF SP can actually be shown to represent an underlying relationship between them. This relationship was previously described as the same *absolute*



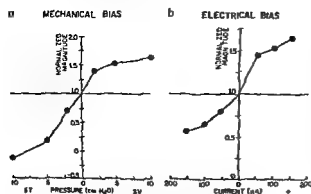


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The studies discussed above leave several questions open. First, is the apparent comparability of electrical and mechanical biasing illustrated by Fig 1, more than coincidence? Secondly, is it equally applicable to both positive and negative components of the SP? Third, are the effects of COCB stimulation on the SP in any way analogous to the effects of electrical and mechanical biasing? In order to resolve these issues, it was necessary to examine the effects of all three forms of biasing on the SP under a variety of stimulus and recording conditions. The details of these investigations have been described elsewhere (Durrant & Dallos, 1972, 1974; Gans, 1974). The purpose of the present writing is to present a direct comparison of the effects of the three forms of biasing on the SP recorded with differential electrodes.

## METHODS

Guinea pigs were utilized throughout the studies. The animals were prepared with differential recording electrodes, generally in the first cochlear turn. The tone burst elicited responses were averaged by means of a digital computer. Since the probe stimuli were phase coherent tone bursts, the microphonic elicited by the probe were cancelled through averaging. The sound stimuli were presented and monitored directly in a closed acoustic system.

Three experimental techniques were utilized to alter the SP, the details of which have been described elsewhere (Durrant & Dallos, 1972, 1974; Gans, 1974). Only a brief description is provided here.

### 1 D C polarization

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Table I *Mechanical and electrical events in the cochlea associated with various parameters of biasing and the cochlear electrical potentials*

Parameter	Corresponding event	
I Mechanical bias		
A Intracochlear pressure	ST pressure increase	SV pressure increase
II Sound	Rarefaction	Compression
II Basilar membrane displacement	Toward SV	Toward ST
VI CM output	Neg phase	Pos phase
VI Electrical bias	Neg Current	Pos Current
V COCB bias	Activated	—
VI EP	Decreased	Increased
VI SP		
A DIF	Decreased	Increased
B DIF <sup>+</sup>	Increased	Decreased
C Direction of change	More pos	More neg

the endocochlear potential (EP). From the effects of the microphonic elicited by the low-frequency biasing stimulus and the expected change in the EP due to the polarization current (Honrubia & Ward, 1969) and COCB stimulation (Fex, 1967) it can be surmized that the effects of all three forms of biasing reflect electrically similar and related events. Table I describes a comparison of the corresponding events underlying all of the experimental procedures reported here, including the mechanical biasing technique of Butler & Honrubia (1963) and other pertinent electrophysiological events. Increased pressure applied to scala tympani and the rarefaction phase of the biasing sound wave both lead to deflection of the basilar membrane toward scala vestibuli. Coincidentally, the negative phase of the microphonic is mirrored by an effective decrease in the EP. Both negative current polarization and COCB stimulation bring about a reduction in the EP. The net effects are such that when a particular biasing condition is associated with a decrease in the EP, the DIF SP responses are made more positive. This is true whether the change in the EP is brought about directly by an externally applied current or indirectly by deformation of

the cochlear partition or hyperpolarizing membrane current flow at the base of the hair cell which presumably results from efferent activity. It follows that when the EP increases, the SP becomes more negative. This can be brought about under electrical or mechanical forms of biasing but not under COCB stimulation.

That there are considerable qualitative similarities between the effects of stimulus, electrical, and COCB biasing on the SP does not mean that they are perfectly analogous. There are various differences between their overall effects, especially as seen in their individual influence upon the cochlear microphonic. Electrical biasing simply causes an increase or decrease in the CM according to the direction of current flow—positive polarization (scala vestibuli positive) enhances, and negative (scala vestibuli negative) depresses the CM. Under stimulus biasing the net effect is typically one of depression of the CM under most conditions or, in other words, an interference effect. COCB stimulation causes the CM to be “paradoxically” increased (Fex, 1967; Sohmer, 1965), and these effects on the CM are limited to certain frequencies (Konishi & Slepian, 1971a). However, even in the face of such diversity underlying similarities prevail. Under all forms of biasing the changes induced in the CM, if any, are much less dramatic than those observed in the SP.

It might be suggested that the three forms of bias incorporated in these studies act at three different levels of the hair cell transduction system. Invoking a slightly modified Davis model (1965), the CM generator may be envisioned as a nonlinear modulated resistance. The following modes of action due to the different biasing procedures may then be conceived: (1) Electrical biasing acts to alter the resting current driven through the modulated resistance. (2) Stimulus biasing serves to alter the quasi-steady state value of the resistance. (3) COCB stimulation, by altering the membrane polarization, influences the load on the resistance. Although the net effect of the biasing would be of the same nature, that is the manipulation of the electrical operating characteris-

tics, the detailed effects would not necessarily be identical due to the different ways in which the bias and the probe might interact under the different biasing paradigms

At this time one can only speculate about such matters, but the results of the studies reported here do offer compelling evidence that there is at least a strong underlying relationship between the effects of electrical polarization, stimulus biasing, and COCB stimulation. By virtue of the presumed selective electrical effects of d.c. biasing and COCB stimulation, it may be concluded that the SP appear to arise from electromechanical nonlinearities. In other words, SP production is dominated by distortion in the hair cell output. Although the underlying mechanisms are still not clearly understood, it would seem that both the DIF<sup>+</sup> and DIF<sup>-</sup> SP components are attributable to this same basic distortion process.

## ZUSAMMENFASSUNG

Unter Anwendung der Differenzialelektroden-technik wurde das summierende Potential (SP) der Meerschweinischnecke aufgezeichnet. Die Auswirkungen der Beeinflussung von der Schneckenkapsel auf das SP wurden unter drei verschiedenen Bedingungen beobachtet:

1. Elektrische Beeinflussung — direkte Stromstimulation, 2. Erregungsbeeinflussung — mechanische Erregung, verursacht durch Niederfrequenz Lautung, 3. COCB Beeinflussung — elektrische Reizung des gekreuzten „olivocochlear“-Bündels. Es zeigte sich, dass alle drei Arten der Beeinflussung ähnliche Auswirkungen auf das SP hatten, die als die „absolute Richtungsänderung“ im Potential bezeichnet werden können. Bei negativer Stromrichtung (scala vestibuli negativ, scala tympani positiv), der negativen Phase der Erregungsbeeinflussung (Ablenkung der Schneckenkapsel zur scala vestibuli) oder bei der Reizung des COCB wird das SP jeweils entweder stärker positiv oder schwächer negativ. Mit anderen Worten, die negative Komponente des SP ist durch die mechanische Verzerrung zu erklären.

scheint auf der mechano-elektrischen Verzerrung zu beruhen, die vom Haarzellenübertragungsprozess hervorgerufen wird.

## ACKNOWLEDGEMENT

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# MACROMOLECULAR TRANSPORT BY THE MIDDLE EAR AND ITS LYMPHATIC SYSTEM

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**Abstract** The macromolecular transport in the middle mucosa of the guinea pig was investigated by means of light and electron microscopy using tracer substances such as Evans blue, India ink, and horseradish peroxidase (HRP). HRP particles were readily taken up by the middle mucosa and Eustachian tube. They were absorbed by all cell types, including ciliated, secretory and non secretory cells. The particles were first taken up by the pinocytotic vesicles and then transported into the intercellular spaces by reverse pinocytosis. These particles were transported toward the connective tissue through

that most antigenic substances that come into contact with the mucosal lining of the respiratory and gastrointestinal tract are macromolecules such as proteins and mucopolysaccharides (Waldman, 1970). The present experiment was undertaken to elucidate the mechanism(s) involved in the uptake and processing of various macromolecules by the mucosa of the middle ear and Eustachian tube, and the regional lymph nodes.

## MATERIALS AND METHODS

Healthy guinea pigs weighing between 400 and 700 gm were used in this experiment. The experimental procedures were performed under general anesthesia with methoxyflurane (Pitman-Moore Co.).

Three tracer substances were used in the absorption studies of the middle ear.

(1) *Evans blue* The Eustachian tubes of 2 guinea pigs were instilled through the middle ear branches with 0.2 ml of Evans blue (Chilcote, Morris Plains, N.J.).

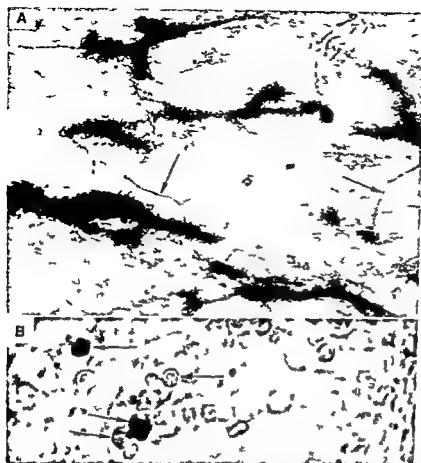
The auditory canal of the guinea pig was filled with dye to prevent leakage. The external canal caused stagnation of the dye in the middle ear. The animals were sacrificed 96 hours after dye instillation. The middle ear was removed and pieces of the middle ear mucosa were stained with 1% osmium tetroxide and traces of glutaraldehyde. The middle ear mucosa and the Eustachian tubes were removed and pieces of the middle ear mucosa were stained with 1% osmium tetroxide and traces of glutaraldehyde. The middle ear mucosa and the Eustachian tubes were removed and pieces of the middle ear mucosa were stained with 1% osmium tetroxide and traces of glutaraldehyde.

HRP in these lymph nodes were mainly found in the specific granules of the macrophages.

The normal middle ear mucosa is known to absorb soluble and particulate matter (Bortnick & Proud, 1965; Breuninger & Feine, 1968; Straube & Westphal, 1971; Haye, 1972). However, only sketchy information is available about the lymphatic drainage from the middle ear in humans (Rouviere, 1932; Graves & Edwards, 1944) and even less in laboratory animals.

Absorption by the mucous membrane has an important bearing on the development of mucosal and general immunity. It is well established and supported in part by the research grants from NIH NDS NS-08854-04 and the Deafness Research Foundation.

<sup>1</sup>On leave of absence from Department of ENT University of Innsbruck, Austria.



**Fig 1** (A) A phase contrast micrograph shows the surface view of the guinea pig middle ear mucosa 30 min after instillation of Evans blue into the bulla. Dark channel streaks are formed by heavy accumulation of the dye. Small blood capillaries are indicated by arrows.  $\times 250$ . (B) A high magnification phase contrast micro-

graph shows numerous tissue macrophages (histiocytes) in the connective tissue layer of the guinea pig middle ear mucosa. Several histiocytes, indicated by arrows, have taken up the dye 60 min after instillation of Evans blue into the bulla.  $\times 250$ .

microscope (Zeiss). The lymph nodes in the neck, which drain the middle ear and auditory tube, were identified by bluish stains. These nodes were removed and routinely processed for histological examination. Whole pieces of the tympanic mucosae were dissected and mounted for surface examination.

(2) *India ink* In 3 guinea pigs 0.2 ml of diluted black India ink (1:3 with sterile normal saline) was injected bilaterally into the tympanic cavities. The animals were kept alive for 24, 48 and 62 hours. The temporal bones and lymph nodes were dissected as described above. The tissue was fixed in formalin and routinely processed for histological evaluation.

(3) *Horseradish peroxidase (HRP)* 0.15 ml of

HRP solution (30 mg HRP in 0.5 ml normal saline) (Type II, Sigma Chemical Company, St. Louis, molecular weight 33 000) was injected into the left tympanic cavities of 14 guinea pigs. The animals were sacrificed after 5, 15, 30 and 60 min following HRP instillation and were perfused intracardially with 2% glutaraldehyde. Pieces of the tympanic mucosa, the Eustachian tube, were dissected in all animals. In 4 guinea pigs the retroauricular and junctional lymph nodes were also removed. The specimens were processed according to the HRP technique as modified by Duvall and Sutherland. The tympanic mucosa and tubes and lymph nodes from the right sides of the animals served as controls.



Fig. 2. A  
r ml.  
te mu  
the epithelium. Fifteen minutes after the introduction of

HRP, the particles were  
(PV) of the ciliated cell  
(B), intercellular space  
layer (CT) BM

## RESULT

middle ear

Evans blue and India ink were both noted in  
the middle ears up to 96 hours after instillation

into the bulla. A  
was accumulated  
of dye were seen  
the nasopharynx  
whole mount

microvilli (MV), micro-  
HRP particles were  
vesicles (PV) 30 min  
RP particles did not  
complex (JC) between  
cell (C)



*Fig. 3* (A) Thirty minutes after instillation into the bulla, the HRP particles were seen in the lysosomal bodies (*Lys.*) of epithelial cells (*Epi.*) and in the lymphatics (*LC*) *BM*, basement membrane; *CT*, connective tissue. (B) Sixty minutes after the bullar instillation, the HRP particles were seen in lysosomal bodies (*Lys.*) of squamous type mucosal epithelium (*Epi.*). Some diffuse HRP particles

were noted in the connective tissue layer (*CT*), but a few particles were found on the surface of the epithelial cells. (C) Thirty minutes after the bullar instillation HRP particles were found in the apex and periphery of a secretory (*S*) cell and in the multivesicular body (*MV*) of a ciliated cell (*C*). *MV*, microvilli.

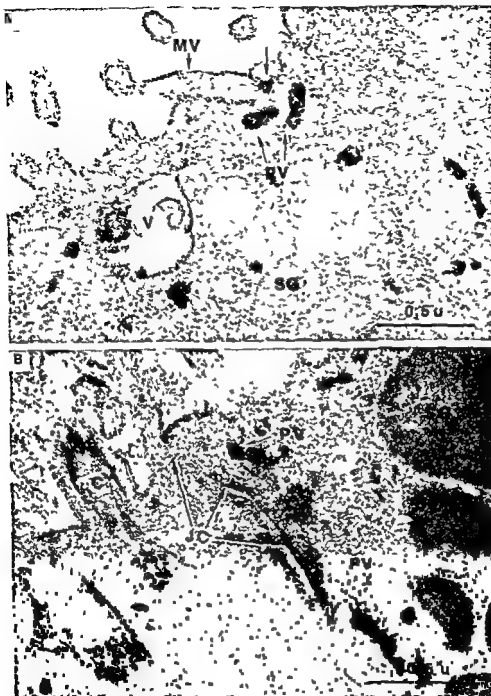
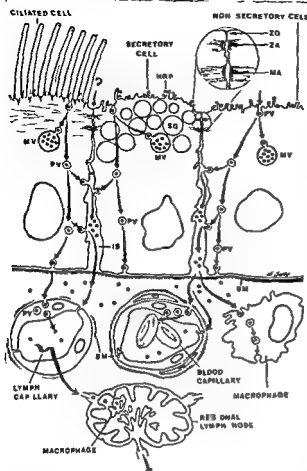


Fig 4 (A) A portion of the free surface of a secretory cell shows numerous pinocytotic vesicles (PV) which contain HRP particles 30 min after instillation with HRP suspension into the middle ear. The HRP particles appear to adhere to the cell surface first and then a pit containing adhered HRP particles is formed at the base of the microvilli (arrow). Some pinocytotic vesicles fuse with a

large vacuole (V) which contains dark bodies. MV, microvilli; SG, secretory granule. (B) The HRP particles were mainly taken up by the pinocytotic vesicles (PV) 30 min after instillation of the bulla. The HRP particles did not pass through the intact junctional complex (JC) between a secretory cell (SG) and a ciliated cell (C).





**Fig 5** A schematic diagram illustrates the mechanisms of cellular transport of macromolecules (HRP) by the middle ear mucosa. The major system for uptake of the molecules by the epithelium is accomplished by pinocytosis (PV) of the cellular membrane facing the ear cavity. Then they are either stored in the multivesicular body (MV) or transported into the intercellular space (IC) or transported across the cell toward the basement membrane (BM). The HRP does not pass through the junctional complex which includes zonula occludens (ZO), zonula adherens (ZA) or macula adherens (MA). However, the HRP readily passes through the basement membrane of the epithelial cells. The tissue macrophages take up large quantities of HRP in the connective tissue layer. Some of the HRP enters into either the lymphatics or blood capillaries by pinocytosis and through the tight junctions. Once the particles enter the lymphatics they are transported into the regional lymph nodes where the lymph macrophages take up most of the transported HRP.

of 2 animals injected with Evans blue showed irregularly arranged blue streaks resembling channels that were independent from the capillary system (Fig 1A). Whether or not these channels represent lymphatic capillaries could not be

determined. India ink particles were found only in the mucous blanket in the middle ear Eustachian tube, some were free and some taken up by macrophages present in the epithelial connective tissue of the middle and Eustachian tube (Fig 1B). Only a scattered India ink particles were detected in lymphatics and blood capillaries.

The tracer particles were found in the epithelial cells and in the subepithelial connective tissue within 5 to 15 min following the instillation of the bulla with HRP (Fig 2). In the specimens, the connective tissue layer was saturated with HRP particles, some of them also seen in lymphatics (Fig 3A). The 60 specimens showed very little HRP particle present on the surface of epithelial cells. The majority of them were found in the connective tissue and in the lysosomal bodies of the epithelial cells (Fig 3B).

Uptake of HRP occurred in all epithelial types including secretory, non-secretory, ciliated cells (Fig. 3B and 3C). The mode of HRP uptake by epithelial cells was accomplished by pinocytosis. First, particles adhered to the surface of microvilli, then the cytoplasm invaginated to form a vesicle containing these particles (Fig 4A). The particles did not pass through the intact tight junctions of the epithelium (Fig 4B). The particles transported by vesicles appeared in (1) intercellular spaces, lysosomes and multivesicular bodies, and (3) connective tissue layer, as illustrated in a schematic drawing (Fig 5). The particles that entered into lysosomes or multivesicular bodies appeared to remain in the cell within the experimental period. There was no evidence, however, that the particles can be trapped at the level of the epithelial basement membrane, previously considered as a barrier for HRP as noted by Haye (1972). The HRP particles that had diffused into the connective tissue appeared to accumulate near the collagen fibers but not near the reticular fibers (Fig 6). Some HRP particles in the connective tissue were also taken up by histiocytes. But large amounts of tracer particles were transported into lymphatics and blood



6 A close up view of the basal portion of the mucosal epithelium shows intense HRP reaction in the intercellular spaces (ICS) in dark bodies (DB) and in the connective tissue. This HRP was observed 30 min after

middle ear instillation with HRP suspension. Heavy accumulation of HRP can be noted near the collagen fibers (CF) but the reticular fiber area (RF) is free of the particles. M: mitochondrion.

capillaries. The passage of these particles across endothelial cells was accomplished mainly by pinocytosis (Fig. 7A), however, some HRP passed through the intact endothelial cell junction.

Special attention was paid to whether squamous epithelial cells of the mucosa transported more HRP than columnar epithelial cells. However, in the present investigation, the differences

in the HRP uptake by these cells were inconsistent and could, therefore, not be evaluated with certainty.

Some of the epithelial cells that seemed to be in a stage of degeneration, picked up large quantities of HRP in their cytoplasm. These cells were either ciliated or secretory and often appeared to be compressed by surrounding ones. The HRP

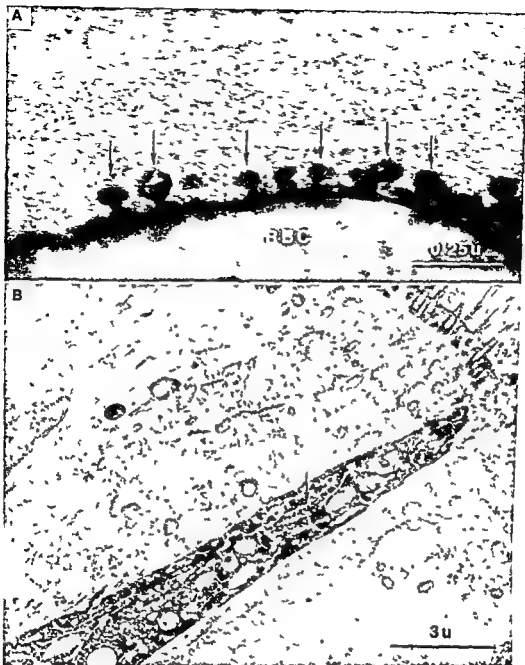


Fig 7 (A) An electron micrograph of the capillary wall of a blood vessel in the middle ear mucosa shows numerous pinocytotic vesicles (arrows). These vesicles contain HRP in the endothelial cell facing the lumen one hour after middle ear instillation with HRP suspension. A

portion of a red blood cell (RBC) is shown (B) accumulation of HRP in an epithelial cell can be seen 30 min after the HRP instillation. On a morphological basis, this cell appears to be a degenerating secretory

reaction products in these cells were distributed diffusely in the cytoplasm and not confined to the cytoplasmic vesicles (Fig 7B).

No cytotoxic effect caused by HRP was noticed in the present experiment, and the peroxi-

dase reaction was not observed in controls: tissues incubated without substrate except in the peripheral part of the red blood cells in capillaries.

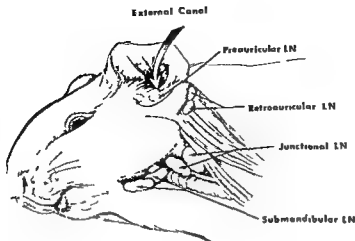


Fig 8 A sketch of the upper cervical lymph nodes of the guinea pig. LN, lymph node. Drawing by Nancy Sally

### Regional lymph nodes

or gross observation, Evans blue yielded the best results for identifying the draining lymph nodes by their pale blue coloration. While the preauricular and submandibular lymph nodes were not stained with Evans blue following middle ear instillation, the retroauricular and junctional lymph nodes (LN) were positively identified as those draining the middle ear and the tube. In two cases, the afferent lymphatics leading into the retroauricular lymph nodes were clearly outlined. The term 'junctional nodes' suggested by Fisch & Sigel (1964) to denote a group of lymph nodes situated below and behind the mandibular angle, as illustrated in Fig 8, was used in the present investigation. Evans blue was not picked up by regional lymph nodes, when only the external ear canal was instilled with dye.

For histological examination, the India ink and HRP yielded the best results. Evans blue could not be detected on unstained sections of those lymph nodes that had macroscopically taken up the dye.

The tracer particles were detected in the ipsilateral retroauricular and junctional lymph nodes as early as 5 min following the tympanic instillation with HRP. More particles were present in the former than in the latter. In the 5 min group, the particles were mainly present in the subcapsular sinuses, but also in capillaries and in the connective tissue of the cortex, mostly

taken up by macrophages (Fig 9A). After longer survival times, macrophages in the medullary part of the lymph nodes had also ingested HRP particles (Fig 9B). The number of HRP ingested macrophages was proportionally increased as the length of the survival time of the animal extended. At the ultrastructural level, reaction products were found to be mainly confined to the specific granules of macrophages (Fig 10).

### DISCUSSION

The present investigation confirmed earlier reports that the middle ear mucosa performs an absorptive function (Bortnick & Proud, 1965; Breuninger & Feine, 1968; Terrahe & Westphal, 1971; Haye, 1972). This function depended largely on the size of the foreign particles, since only a few carbon particles of India ink, with an average diameter of 400 Å, were taken up, whereas, HRP particles (diameter 50–60 Å) readily passed through the epithelium. Therefore, the mode of absorption and transcellular transport of macromolecules was mainly studied using HRP as a tracer.

It is now established that the epithelium of normal middle ear mucosa of human and animal alike consists of ciliated, secretory and non-secretory cells and that the latter, in general, are squamous cells possessing varying numbers of microvilli on their surfaces (Sade, 1966; Lim et al., 1967; Kawabata & Paparella, 1969; Hussel



Fig. 9 (A) A phase contrast micrograph of a junctional lymph node 15 min after HRP instillation of the bulla. The black deposits (HRP reaction) are mainly in the macrophages of the subcapsular area and in the macrophages of the cortex. Some HRP particles can be seen in

the sinus.  $\times 250$  (B) Thirty minutes follow instillation of the bulla, the particles were abundant in many of the macrophages located in the medulla of the junctional lymph nodes.  $\times 250$

& Lim 1969; Hentzer 1970; Kaneko et al. 1971; Hilding & Heywood 1971; Lim et al. 1972; Shimada & Lim 1972).

The present experimental evidence showed that absorption and transcellular transport of HRP was accomplished by all epithelial cell types. The HRP particles were taken up by epithelial cells with pinocytosis and then mainly

transported to the intercellular space by pinocytosis, although some of them were up taken by lysosomes and multivesicular bodies. This absorptive mechanism is similar to that of body transport across the proximal absorptive cells in the small intestine of the neonate (Rodewald 1973) and that of ferritin transport across Reissner's membrane (Hinojosa

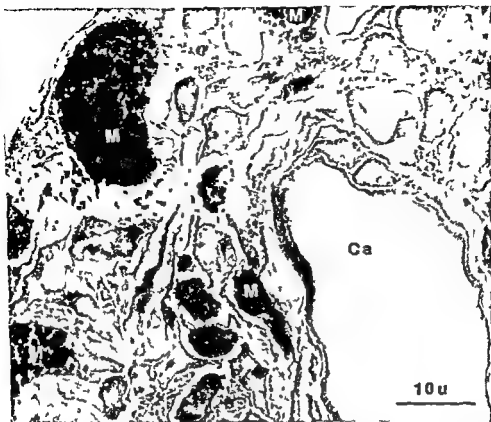


Fig. 10 Among numerous lymphocytes several macrophages (M) containing HRP can be seen in the junctional

lymph nodes of the guinea pig 15 min after bullae instillation with HRP suspension. Ca capillary

We could not confirm the earlier report that the tracer particles passed through the tight junctions of the epithelial cells (Terrahe & Westphal 1971; Haye 1972). The HRP particles freely passed through the intact epithelial basement membrane which did not constitute a barrier to the diffusion of HRP as noted by Haye (1972).

The collagenophilic and reticulophobic properties of the tracer particles (HRP) observed in the present experiment could not be explained, however, due to this peculiarity patchy accumulation of the tracer particles was noted in the connective tissue layer.

The mechanism involved in transport of HRP particles across the endothelial cells of the lymph and blood capillaries in the middle ear was similar to that in the capillaries of stria vascularis (Duvall & Sutherland 1972) where the tracer particles were mainly transported by pinocytosis and reverse pinocytosis. But also some particles

passed through the tight junction of the endothelial cells as observed by Karnovsky (1967) in the vessels of skeletal and cardiac muscles.

Although the precise route of the draining lymphatics from the tympanic cavity and the tube were not clearly demonstrated in this study the present finding strongly suggests that retroauricular and junctional lymph nodes are the major lymph drainage system for these areas in the guinea pig. This finding is in agreement with the report by Arnold et al. (1972). They observed small particles injected into the subarachnoid space were found in the submucosal connective tissue of the middle ear and drained by the regional lymph node (retro-auricular node). Their finding confirmed an earlier experiment conducted by Karbowski (1930) also using guinea pigs. The regional lymph nodes in our study were stained as early as 5 min after instillation of Evans blue into the bulla. In the

human, the lymphatics of the middle ear and Eustachian tube are thought to drain to the retropharyngeal, pharyngeal, parotid, submandibular and upper deep cervical lymph nodes (Rouviere, 1932, Graves & Edwards, 1944). Preauricular, parotid, submandibular and jugular lymph nodes were not stained with Evans blue in the present experiment. The retropharyngeal lymph node described in the human could not be identified in the guinea pig.

It is known that histiocytes (tissue macrophages) are present in normal middle ear mucosa, in infected ears (Lim & Klainer, 1971) and in ears with serous otitis media (Lim & Birck, 1971, Lim et al., 1972). While it is apparent that histiocytes engulf a large amount of HRP particles in the middle ear, there is no evidence that the number of histiocytes increased as the result of HRP instillation. However, in the regional lymph nodes, the number of macrophages appeared to increase in direct proportion to the length of time between the HRP instillation and the sacrifice of the animal. In 5-min specimens, the HRP particles were mainly present in macrophages in the subcapsular sinuses. But in the 30- and 60 min groups, an increasing number of macrophages in the medullary area had taken up the tracer particles. This finding suggests that as the lymph reaches the subcapsular sinuses through the afferent lymphatics, part of the transported particles are ingested by macrophages, and as the lymph passes through the node from the cortical to the medullary areas, more particles are phagocytized by macrophages. We can speculate that the excess tracer particles will then be carried from the lymph node via the efferent lymphatics to the next lymph nodes.

Mucosal immunity is based upon the presence of a local immunological defense system in the mucous membranes of the respiratory and gastro-intestinal tract. This concept had been postulated by Besredka in 1927, but was substantiated only in the last decade (Waldman, 1970, Tomasi, 1972). Recent evidence has shown that the middle ear mucosa is also endowed with such a defense system (Bernstein et al., 1972,

Ishikawa et al., 1972, Lim et al., 1972, Mogal, 1973). A prerequisite for the function of this system is that foreign substances (macromolecules) can be absorbed by the lining mucosa and then processed by macrophages. The processed substances can act as antigens to stimulate antibody-forming cells (Pearsall & West, 1970).

The present experiment and the results of other investigators (Terrahe & Westphal, 1971, Haye, 1972) confirm that the middle ear mucosa can take up large quantities of macromolecules and can process them by macrophages present in the mucosal level and also in the regional lymph nodes. This finding further supports the notion that the middle ear is provided with an immunological defense system.

## ACKNOWLEDGEMENT

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## ZUSAMMENFASSUNG

Unter Anwendung verschiedener Tracer Substanzen (Evans Blau Tusche, Meerrettich Peroxidase) die Meerschweinchen durch das Trommelfell in Pauke Bulla instilliert wurden, konnte die Resorptionsleistung der gesunden Mittelohrschleimhaut und der Abtransport der Markierungssubstanzen in die regionalen Lymphknoten auf licht und elektronen mikroskopischer Ebene untersucht werden. Während Tusche und Peroxidase sich als geeignete Markierungssubstanzen für histologische bzw. ultrastrukturelle Untersuchungen wiesen, diente Evans Blau der makroskopischen Identifizierung der abhängigen Lymphknoten.

Die resorptive Leistung der Mittelohrschleimhaut hängt von der Grösse der zu resorbierenden Partikel. Während Tusche Teilchen (Durchmesser etwa 400 nm) nur in geringem Masse in das Epithel aufgenommen wurden, Grossteil aber durch die Tube abtransportiert wurden, wurden Peroxidase Partikel (Durchmesser 50-60 nm) rasch von der Schleimhaut resorbiert. Das Tracer Protein wurde von allen Epithelzell Typen durch Pinocytose aufgenommen und vorwiegend in die Interzellularlücken weitertransportiert. Während die epithelialen Schleimhäute eine Barriere für das Eindringen des Tracer Proteins darstellen, kann dieses die Basalmembran des Epithels ohne Schwierigkeiten passieren. Im subepithelialen Bindegewebe wurde das Reaktionsprodukt von einer kollagenen Fasern in Histiozyten und in grosser Menge in Blut und Lymphkapillaren festgestellt.

Bereits 5 Minuten nach Instillation von Peroxidase-  
 pillaren und Makrophagen des Kortextbereiches fand,  
 te sie sich nach längeren Überlebenszeiten nach 30  
 nuten und mehr vor allem in Makrophagen in den  
 atralen Abschnitten der Lymphknoten

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## PROTEINKONZENTRATION DER MEERSCHWEINCHEN-PERILYMPHE NACH SCHALLBELASTUNG

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(Eingegangen am 27. Juni, 1974)

**Abstract** Die Proteinkonzentration der Perilymphe larmbelasteter Meerschweinchen wurde mit Hilfe einer früher beschriebenen Mikromethode untersucht. Die Tiere wurden einseitig in einem akustisch geschlossenen System 1 Stunde mit Breitbandrauschen der Intensität von 140 dB beschallt. Die Perilymphe wurde zu verschiedenen Zeiten nach Beschallungsende entnommen. Sofort nach 6 und 24 Stunden. Bei den beschallten Tieren ist es wesentlich problematischer, blutfreie Perilymphe zu gewinnen als beim Normaltier. Selbst post mortem ist es schwierig, blutfreie, nicht hämolytische Proben zu gewinnen, besonders aus der Scala tympani. Ein Teil der Tiere wurde deshalb vor dem Dekapitieren intraarteriell mit Ringer-Lösung bzw. Infukoll® 40 perfundiert. Nach der Beschallung ist nur bei den intraarteriell perfundierten Tieren eine geringe Erhöhung der Proteinkonzentration in der Perilymphe der beschallten Ohren nachweisbar. Der Konzentrationsunterschied ist bei den 6 Stunden nach Beschallungsende entnommenen Proben am deutlichsten. Ein sicherer Hinweis auf die Herkunft dieses Proteins konnte aus den Untersuchungen nicht gewonnen werden.

Unsere früheren Untersuchungen (Scheibe et al., 1972) haben gezeigt, daß eine qualitative Änderung des Proteinmusters der Perilymphe (PL) nach hörschädigender Schallbelastung immunoelektrophoretisch nicht nachweisbar ist. Das schließt nicht aus, daß sich die Proteinkonzentration der PL durch die Beschallung ändert. Bisher wurden dazu nur orientierende Untersuchungen an Kaninchen (Miyake, 1960) und Katzen (Komarovitch & Plouzhnikov, 1966) mit unterschiedlichen Ergebnissen durchgeführt. Wir haben deshalb unsere qualitativen Untersuchungen

larmbelasteter Meerschweinchen quantitative Untersuchungen ergänzt. Grundlage dienten vorangegangene Untersuchungen am normalen unbeschallten Tier (Scheibe et al., 1974). Ziel der jetzigen Untersuchung ist die Klärung der Fragen:

1. Ändert sich nach hörschädigender Belastung die Proteinkonzentration der PL?
2. Welche Rückschlüsse lassen sich aus solchen Konzentrationsänderungen auf Schädigungsmechanismen ziehen?

### MATERIAL UND METHODEN

#### Gewinnung der PL

Die Untersuchung wurde mit 200–300 g schweren Meerschweinchen (Prüfung des Preyerschen Ohrmuschelreflex) in Äthernarkose durchgeführt. Die PL-Gewinnung erfolgte in der schon beschriebenen Weise (Scheibe et al., 1975) unmittelbar post mortem und am lebenden Tier nach verschiedenen Methoden.

#### Beschallung der Tiere

Für die Belastungsversuche wurden die Tiere einseitig im akustisch geschlossenen System (Wagner et al., 1974) 1 Stunde mit Breitbandrauschen der Intensität von 140 dB beschallt. Unter einem akustisch geschlossenen System verstehen wir die schalldichte Ankopplung eines dynamischen Meßschallwandlers an den

Die Untersuchungen wurden im Auftrage des Ministeriums für Gesundheitswesen der DDR im Rahmen der Lärmschadenforschung durchgeführt.

Abbildung 1  
Tabelle I  
Proteinkonzentration (mg/100 ml) von  
nach Perfusion mit Ringer-Lösung postmortal  
gewonnener Gesamt-Perilymphe einseitig be-  
schallter Tiere

Perilymphe Entnahme nach Beschallung	Kontralaterales Ohr	Beschalltes Ohr
Sofort	146 ± 29 (12)	174 ± 53 (12)
nach 6 Stunden	139 ± 24 (10)	176 ± 44 (10)
nach 1 Tag	154 ± 40 (9)	150 ± 39 (9)

ernen Gehörgang des Meerschweinchens mit  
Hilfe eines Trichters. Bei den angewendeten  
Schallungsparametern ist mit einer starken  
Schädigung des Haarzellbestandes auf der  
gesamten Basilarmembran zu rechnen. Die PL  
wurde zu verschiedenen Zeiten nach Beschal-  
lungsende entnommen.

#### Proteinbestimmung und Auswertung

Die Proteinbestimmung erfolgte nach Lowry  
et al. (1951) in der schon beschriebenen Mikro-  
modifikation (Scheibe et al., 1974).

Innerhalb der einzelnen Versuchsreihen wurde  
die PL-Proteinkonzentration der beschallten  
Ohren mit der der kontralateralen Ohren ver-  
glichen. Da die kontralateralen Ohren der  
einseitig beschallten Tiere nicht als unbeschallt  
betrachtet werden können, erfolgte außerdem ein Vergleich  
der Proteinwerte mit denen normaler un-  
beschallter Tiere (Scheibe et al., 1974). Die  
Bewertung der Mittelwerte wurde mittels t-Test  
durchgeführt.

## ERGEBNISSE

### PL-Gewinnung

Nach Schallbelastung ist die intravitale Gewinn-  
ung blutarmer PL, besonders aus der Scala  
media, noch wesentlich schwieriger als bei  
Normaltieren. Die sonst vorteilhafte Entnahme  
der tympanalen Proben durch das runde Fenster  
fällt bei den beschallten Ohren ungünstig aus. Das  
runde Fenster erscheint makroskopisch in den  
meisten Fällen stark gerötet. Unter dem Opera-  
tionsmikroskop erkennt man eine deutliche  
Gefäßverengung. Dadurch läßt sich bei der

Punktion der runden Fenstermembran eine  
Gefäßverletzung und damit eine Blutverun-  
reinigung der PL kaum vermeiden. Nach  
Verdünnung des Knochens ist mikroskopisch  
auch die Erweiterung der Gefäße an der lateralen  
Skalenwand zu erkennen. Deshalb ist auch bei  
Punktion des Knochens die Ausbeute an blutar-  
men Proben wesentlich geringer als bei Normal-  
tieren.

Bei der postmortalen PL-Gewinnung ist  
ebenfalls häufiger mit Blutverunreinigung der  
Proben zu rechnen. Nur nach intraarterieller  
Perfusion der Tiere ist auch von beschallten  
Ohren in den meisten Fällen nahezu blutfreie  
PL zu gewinnen.

### Proteinkonzentration von nach Perfusion mit Ringer-Lösung postmortal gewonnenen Gesamt-PL beschallter Tiere

In Ergänzung unserer bisherigen qualitativen  
Untersuchungen (Scheibe et al., 1972) wurde  
auch bei den jetzigen Belastungsversuchen  
zunächst Gesamt-PL von mit Ringer-Lösung  
perfundierten Tieren untersucht. Die PL-Proben  
beider Ohren (beschallt, kontralateral) wurden  
zu verschiedenen Zeiten nach Beschallungs-  
ende entnommen. Sofort, nach 6 Stunden und nach  
1 Tag. Die Ergebnisse dieser 3 Gruppen sind  
in Tab. I zusammengestellt. Daraus geht hervor,  
daß bei den sofort (1. Reihe) und bei den nach  
6 Stunden (2. Reihe) gewonnenen Proben die  
Proteinwerte der beschallten Ohren etwas höher  
liegen als bei den kontralateralen Ohren. Der  
Konzentrationsunterschied ist (bei dem unter-  
suchten Stichprobenumfang) aber nur bei den  
nach 6 Stunden entnommenen Proben signifi-  
kant ( $P < 5\%$ ). Bei den nach 1 Tag entnom-  
menen Proben ist kein Unterschied nachweisbar.

Da die PL-Proteinkonzentration der kon-  
tralateralen Ohren zunächst nicht als normal,  
d. h. von der Beschallung unbeeinflusst, angesehen  
werden kann, haben wir diese Werte mit denen  
normaler unbeschallter Tiere verglichen. Die  
am Normaltier gewonnenen Ergebnisse (Tab. II)  
wurden in einer vorangegangenen Arbeit (Scheibe  
et al., 1975) bereits mitgeteilt. Für die Gesamt-  
PL fanden wir eine mittlere Proteinkonzentra-

**Tabelle II** Proteinkonzentration (mg/100 ml) von mit unterschiedlicher Methodik postmortal gewonnener Perilymphe unbeschallter Tiere

Probengewinnung	Vestibuläre Perilymphe	Tympanale Perilymphe
Sofort postmortal	154 ± 57 (27)	208 ± 70 (15)
Nach Perfusion mit Infukoll M 40	133 ± 36 (16)	150 ± 28 (13)
Nach Perfusion mit Ringer Lösung	Gesamt Perilymphe 159 ± 40 (37)	

tion von  $159 \pm 40$  mg/100 ml ( $n=37$ ). Dieser Mittelwert unterscheidet sich nicht signifikant von den Mittelwerten der kontralateralen Ohren der einseitig beschallten Tiere (Tab I). Dadurch ergeben sich auch zwischen beschalltem und unbeschalltem Ohr bzw. Tier dieselben geringen Konzentrationsunterschiede wie zwischen beschalltem und kontralateralem Ohr der einseitig beschallten Tiere. Wir haben deshalb auch bei den weiteren Belastungsversuchen die Tiere einseitig beschallt und zunächst das beschallte mit dem kontralateralen Ohr verglichen. Zusätzlich erfolgte auch hier noch ein Vergleich mit den analytischen Daten der unbeschallten Tiere (Tab II).

**Proteinkonzentration von postmortal gewonnener vestibulärer und tympanaler PL nach Schallbelastung**

Bei funktionellen Untersuchungen sind Konzentrationsänderungen in der PL, die durch Stoffwechseländerung oder Zellschädigung des Cortischen Organs bedingt sein können, in stärkerem

Maße in der tympanalen als in der vestibulären PL zu erwarten. Die vorangegangenen Untersuchungen am Normaltier (Scheibe et al 1) haben aber gezeigt, wie problematisch gerade Gewinnung der tympanalen PL ist. Auch subokzipital eröffnetem Liquorraum ist die tympanalen Proteinwerte relativ stark. Den beschallten Tieren ist es außerdem besonders schwierig, die tympanale PL intrakraniell blutfrei zu gewinnen. Wir haben deshalb den weiteren Belastungsversuchen postmortal gewonnene PL untersucht.

Die PL wurde zuerst durch die übliche Funktion der herausgebrochenen Schnecke sofort bzw. 6 Stunden nach Beschallung gewonnen. Obwohl auch bei diesen Versuchsreihen nur PL mit einer Blutverunreinigung  $<0,25\%$  verwendet wurde, erschienen tympanale Proben, und zwar sowohl von beschallten als auch von den kontralateralen Ohren, nach dem Zentrifugieren häufig etwas hämolytisch. Von den sofort nach der Beschallung entnommenen tympanalen Proben waren ungefähr 25–30% etwas hämolytisch. Sie wurden nicht in die Berechnung mit einbezogen. Ergebnisse dieser Versuchsreihe sind in Tab I (1. Reihe) dargestellt. Sie zeigen, daß sich Proteinwerte der beschallten und kontralateralen Ohren unmittelbar nach der Beschallung nicht unterscheiden, weder von vestibulärer noch von tympanaler PL.

Von den 6 Stunden nach Beschallung entnommenen tympanalen Proben (Tab I, 2. Reihe) waren bei den kontralateralen Ohren ungefähr 20% etwas hämolytisch und wurden verworfen. Bei den beschallten Ohren waren

**Tabelle III** Proteinkonzentration (mg/100 ml) von mit unterschiedlicher Methodik postmortal gewonnener vestibulärer und tympanaler Perilymphe einseitig beschallter Tiere

Perilymph Entnahme nach Beschallung	Vestibuläre Perilymphe		Tympanale Perilymphe	
	Kontralaterales Ohr	Beschalltes Ohr	Kontralaterales Ohr	Beschalltes Ohr
Sofort	136 ± 42 (21)	138 ± 51 (21)	152 ± 50 (13)	159 ± 46 (11)
Nach 6 Stunden	153 ± 37 (10)	159 ± 38 (13)	186 ± 75 (7)	240 ± 77 (8)
Nach 6 Stunden, Perfusion mit Infukoll M 40	185 ± 42 (28)	208 ± 56 (28)	163 ± 42 (20)	171 ± 51 (19)

wegen ungefähr 75% der tympanalen Proben hämolytisch. Dadurch wäre für einen genügend großen Stichprobenumfang von blutarmen nicht hämolytischen Proben ein sehr hoher Aufwand an Tiermaterial erforderlich gewesen. Wir haben deshalb für eine orientierende Abschätzung die hämolytischen Proben zunächst in die Berechnung mit einbezogen. Das bedeutet, daß bei einer maximalen Blutverunreinigung von 25% die Proteinkonzentration der tympanalen Mittelwerte durch Hamolyse bis zu ungefähr 1 mg/100 ml artifiziell erhöht sein kann. Dadurch ist auch der tympanale Mittelwert (103 ± 77) artifiziell erhöht und wurde deshalb in Tab. III (2. Reihe) in Klammern gesetzt. Die Aussage über einen signifikanten Konzentrationsanstieg in der tympanalen PL der beschallten Ohren ist daher bei dieser Versuchsreihe nicht möglich. Die vestibulären Proteinwerte der beschallten (159 ± 38) und der kontralateralen Ohren (153 ± 37) unterscheiden sich jedoch bei dieser Versuchsreihe nicht. Bei beiden Versuchsreihen ändert sich das Untersuchungsergebnis nicht signifikant, wenn man die Proteinwerte der beschallten Ohren mit den entsprechenden Werten der unbeschallten Tiere (Tab. II, 1. Reihe) vergleicht.

*Proteinkonzentration von nach Perfusion mit Infukoll® M 40 postmortal gewonnener vestibulärer und tympanaler PL beschallter Tiere*

Für abschließende Belastungsversuche möglichst blutfreie PL zu erhalten, haben wir in der letzten Versuchsreihe die beschallten Tiere mit Dekapitieren intraarteriell mit Infukoll® M 40 perfundiert. Auf Grund der bisherigen Untersuchungsergebnisse wurde die Untersuchung 6 Stunden nach Beschallungsende entnommene Proben beschränkt. Trotz der Perfusion waren ungefähr 20–30% der tympanalen beschallten und kontralateralen Proben hämolytisch und wurden verworfen. Die Proteinwerte sind in Tab. III (3. Reihe) enthalten. Sie zeigen, daß sich bei dieser Versuchsreihe die tympanalen Mittelwerte der beschallten (171 ± 51) und kontralateralen Ohren (163 ± 42) kaum

unterscheiden. Bei der vestibulären PL liegt dagegen die Proteinkonzentration der beschallten Ohren (208 ± 56) etwas höher als bei den kontralateralen Ohren (185 ± 42) und bei Paarvergleich ergibt sich Signifikanz für  $P=5\%$ . Auch bei dieser Versuchsreihe ändert sich die Aussage nicht, wenn man für den Vergleich wieder die Proteinwerte der unbeschallten Tiere (Tab. II, 2. Reihe) heranzieht.

## DISKUSSION

Die analytischen Ergebnisse zeigen, daß nach stark schädigender Schallbelastung nur geringe Konzentrationsänderungen der PL-Proteine bei den postmortal gewonnenen Proben der intraarteriell perfundierten Tiere nachweisbar sind. Der Konzentrationsunterschied ist bei den 6 Stunden nach Beschallungsende entnommenen Proben am deutlichsten. Hier fanden wir in der Gesamt PL der mit Ringer-Lösung perfundierten Tiere (Tab. I, 2. Reihe) und in der vestibulären PL der mit Infukoll® M 40 perfundierten Tiere (Tab. III, 3. Reihe) bei den beschallten Ohren eine signifikante ( $P=5\%$ , Paarvergleich) höhere Proteinkonzentration als bei den kontralateralen Ohren.

Bei den nicht perfundierten Tieren (Tab. III, 2. Reihe) ist eine Konzentrationserhöhung in der vestibulären PL der beschallten Ohren bei dem untersuchten Stichprobenumfang nicht nachweisbar. Der erhöhte tympanale Proteinwert der beschallten Ohren ist wahrscheinlich durch Hamolyse in den Proben bedingt. Bei dieser Versuchsreihe müßte für eine eindeutige Aussage der Stichprobenumfang insgesamt erhöht werden. Dazu wäre aber wegen der geringen Ausbeute an blutarmen nicht hämolytischen Proben, besonders bei der tympanalen PL der beschallten Ohren, ein sehr hoher Aufwand an Tiermaterial erforderlich.

Am lebenden Tier, bei dem die Gewinnung blutfreier PL noch schwieriger ist als postmortal, sind Belastungsversuche mit größerem Stichprobenumfang (bei Schallintensitäten von 140 dB) praktisch kaum durchzuführen. Hinzu kommt hier bei den tympanalen Proben die

mogliche Verfälschung durch Liquor. Nach unseren Untersuchungen am Normaltier (Scheibe et al., 1975), bei denen sich die Proteinwerte intravital und postmortal gewonnener Proben nicht signifikant unterscheiden, sind aber auch am lebenden Tier keine wesentlich anderen Belastungsergebnisse zu erwarten.

Der Vergleich mit den Proteinwerten der unbeschallten Tiere (Tab II) zeigt, daß die PL-Proteinkonzentration der kontralateralen Ohren durch die einseitige Beschallung der Tiere nicht signifikant verändert wird. Zwischen beschalltem und unbeschalltem Ohr bzw. Tier ergeben sich daher auch nur dieselben geringen Konzentrationsunterschiede wie zwischen beschalltem und kontralateralem Ohr der einseitig beschallten Tiere.

Eine Erklärung für die etwas höhere Proteinkonzentration in der PL der beschallten Ohren kann bisher nicht gegeben werden. In Betracht käme hauptsächlich ein Proteinaustritt aus schallgeschädigten Zellen des Cortischen Organs (Spoendlin & Brun, 1973) und eine erhöhte Proteinpermeabilität der Kochleagefäße.

Die Belastungsversuche an Kaninchen (Miyake, 1960) und Katzen (Komarovitch & Plouzhnikov, 1966) ergaben unterschiedliche Ergebnisse. Miyake berichtet lediglich, ohne Angaben von Analysenwerten und Versuchsbedingungen, daß nach Beschallung eine Abnahme der Proteinkonzentration in der PL eintritt. Komarovitch & Plouzhnikov haben in 2 Versuchsreihen je 6 Tiere 20–60 Minuten mit einer Sirene (Gesamtschallpegel 140 dB, Grundschwingung 400 Hz) belastet und die PL 30 Minuten bzw. 3 Stunden nach Beschallungsende untersucht. Die Proteinwerte lagen bei Entnahme nach 30 Minuten niedriger und bei Entnahme nach 4 Stunden höher als bei unbeschallten Kontrolltieren. Statistisch gesichert wurden diese Angaben nicht. Als Ursache der Konzentrationsabnahme wird eine Permeabilitätsänderung der Reißnerschen Membran vermutet. Die Konzentrationserhöhung wird als Folge eines nachfolgenden kompensatorischen Proteinaustrittes aus Kochleagefäßen angesehen.

Die vorliegenden Ergebnisse bestätigen unsere

früher geäußerte Annahme (Scheibe et al., 1975), daß es bei funktionellen Untersuchungen schwierig ist, geringe Konzentrationsänderungen in der PL eindeutig festzustellen. Bei Liquorverunreinigung der PL sowie individuellen Links-Rechts-Unterschieden der Proteinwerte machen selbst bei einseitiger Beschallung der Tiere und paarweisem Vergleich beschallten und unbeschallten Ohren eindeutige Aussagen über geringe Konzentrationsänderungen der PL-Proteine problematisch. Es ist daher auch verständlich, daß bei unseren früheren immunoelektrophoretischen Untersuchungen (Scheibe et al., 1972) nach Beschallung keine Änderung der PL-Proteine nachweisbar war.

Zusammenfassend lassen sich die als Zielung formulierten Fragen wie folgt beantworten:

1. Selbst stark hörschädigende Schallbelastung verursacht nur geringe Konzentrationsänderungen der PL-Proteine. Ihr eindeutiger Nachweis ist problematisch.
2. Rückschlüsse auf einen Schallschädigungsmechanismus können aus den Ergebnissen nicht gezogen werden.

## SUMMARY

The protein concentration in the perilymph of noise-exposed guinea pigs was investigated using a method described previously. The animals were exposed to wide band noise at 140 dB for one hour unilaterally in a closed acoustic system. Perilymph was obtained exposure at different intervals: immediately, 6 hours and 48 hours later. From animals exposed to noise, it is particularly more difficult to obtain perilymph without contamination than from normal ones. Even post mortem it is difficult to obtain samples without blood contamination and without hemolysis, particularly from the tympani. Therefore, some animals were perfused arterially with Ringer solution or with Infukoll before decapitation. After exposure to noise only a small increase in the protein concentration of the perilymph can be detected in the noise-exposed ears of animals perfused intra-arterially. The difference in concentration is most distinct in samples extracted 6 hours after exposure had ceased. No reliable clue to the source of this protein could be obtained from these investigations.

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## VERBINDUNGSKOMPLEXE AN ZELLEN DER REISSNER-MEMBRAN IN GEFRIERGEBOCHENEN PRÄPARATEN\*

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(Eingegangen am 20. September, 1974)

**Abstrakt** Die Reissner Membran aus dem Innenohr von Chinchillas wurde mit Hilfe der Gefrierersatzungsmethode untersucht. Zonulae occludentes schließen endolymphwärts den Raum zwischen den Epithelzellen ab. Sie bestehen aus 2 bis 8 übereinanderliegenden Kammen bzw. Talern und ähneln somit morphologisch denen zwischen den Marginalzellen der Stria vascularis, sind jedoch wesentlich schwächer ausgebildet als die zwischen den Basalzellen der Stria.

Die Endolymph unterscheidet sich von der Perilymphe chemisch durch einen höheren  $[K^+]$ - und niedrigeren  $[Na^+]$  Gehalt (Smith et al., 1954, Johnstone et al., 1963), elektrophysiologisch ist ein höheres positives Potential der *scala media* gegenüber der *Scala vestibuli* bzw. *tympani* meßbar (v. Bekesy, 1952, Johnstone, 1967).

Hohe Konzentrations- und Potentialunterschiede werden von Epithelien mit physiologisch dichten Verbindungskomplexen, sogenannten „tight junctions“, aufrechterhalten (Frömter & Diamond, 1972). Claude & Goodenough (1973) fanden den morphologischen Aspekt der „tight junctions“, wie er in Abdrücken gefriergebrochener Präparate darstellbar ist, korrelierbar

mit dem elektrischen Widerstand des jeweiligen Epithelverbandes. In dieser Hinsicht habe die Reissner-Membran mit der Gefrierersatzungsmethode untersucht.

### MATERIAL UND METHODE

Sieben erwachsene Chinchillas wurden mit 100 mg/kg Natrium-Pentobarbital endoperi-anaesthetisiert und die Innenohren durch lymphatische Perfusion vorfixiert. Danach liierten wir die Cochlea, entfernten den Saccus und setzten die Fixierung für weitere vier Stunden fort. Während dieser Zeit haben wir die chierne Kapsel der Cochlea entfernt, den Ductus cochlearis abgetrennt und in einem Fall eine gezielte Präparation — die Reissner-Membran isoliert. Die Fixierungslösung enthielt 2% Formaldehyd und 1% Glutaraldehyd in 0,1 M Cacodylatpuffer (pH 7,3 — 7,4) mit 25 mM  $CaCl_2$ . Nach beendeter Fixierung wurden die Präparate 90 Minuten mit 30% Glycerin in Ringerlösung durchtränkt, auf Präparatenträgerplättchen orientiert, in flüssigem Frost eingefroren und in einem Balzers Gerät 84 M gebrochen sowie mit Platin Kohle bedeckt (Moor & Mühlethaler, 1963). Die Abdrücke reinigten wir in Chromsäure und Natrumpyrophosphat, ließen sie auf Dowell-Membran trocknen und untersuchten sie in Siemens Elektronenmikroskopen Ia und 10I.

\* Vorgetragen auf der 45. Jahresversammlung der Deutschen Gesellschaft für Hals Nasen Ohren Heilkunde Kopf und Hals Chirurgie (Bad Reichenhaller) 26. — 30.5.1974.

Mit Unterstützung des Consiglio Nazionale delle Ricerche (Nr. CT 72.00775.04.115.227 und 71.00863.04.115.1144).



Abb 1 Abdruck einer gefriergebrochenen Reissner-Membran P = Perilymphraum Bm = Basalmembran, Ed = Endolymphraum. Die Pfeile weisen auf die Zonula

occludens der Epithelzellen. Die Pfeilspitze gibt die Bedampfungsrichtung an  $\times 50\,000$

## ERGEBNISSE

Die Abdrücke der gefriergebrochenen Präparate der Reissner-Membran lassen die unter anderem im Meerschweinchen im Dünnschnitt von Nagaiwara (1963) Iurato (1967a, b), Duvall &

Rhodes (1967), v Ilberg (1968) und Duvall & Sutherland (1970) beschriebenen Strukturen erkennen.

Die beiden Zellschichten ektodermaler und mesenchymaler Herkunft sind beim Chinchilla deutlich sichtbar (Abb 1). Die endolymphseitig



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\* Vorgetragen auf der 45. Jahresversammlung der Deutschen Gesellschaft für Hals Nasen Ohren Heilkunde Kopf und Hals Chirurgie (Bad Reichenhaller 26 — 30.5.1974).

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gelegenen Epithelzellen bilden eine vollständige Schicht, die auf der nur teilweise erkennbaren Basalmembran aufliegt. Sie tragen kurze Mikrovilli und zeigen im Zytoplasma einen ausgedehnten Golgi-Apparat, Mitochondrien und Zisternen des endoplasmatischen Retikulums, vorwiegend lateralwärts liegen zahlreiche Mikropinozytosevesikel. Die Plasmamembranen benachbarter Zellen verlaufen unmittelbar unterhalb des Verbindungskomplexes meistens geradlinig, werden dann leicht gewunden und bilden schließlich in der Nähe der Basalmembran Falten.

Im Bereich des Verbindungskomplexes sind Kamme (Fläche A der Zellmembran) und Taler (Fläche B der Zellmembran) sichtbar und sind miteinander anastomosiert und bilden ein unregelmäßiges Netzwerk (Abb 1 und 2). Kamme und Täler stellen Kontaktlinien zwischen benachbarter Zellmembranen dar. Morphologisch entsprechen sie der Zonula occludens, die im gleichen Bereich anderer Epithelien monstriert wurde (Kreutziger, 1968, Staehelin et al., 1969, Chalcraft & Bullivant, 1970, Iurato & Gilula, 1972, Claude & Goodenough, 1973).

Die Zahl der Kamme bzw Täler variiert zwischen zwei (Abb 1) und acht (Abb 2d). Sogenannte „gap junctions“ konnten wir lang im Epithel der Reissnerschen Membran nicht finden.

Die bindegewebigen, flachen Zellen der peripherwärts gelegenen Schicht der Reissner-Membran lassen auch in Abdrücken gefriergebrochener Präparate häufig weite Untersuchungen erkennen. Infolgedessen ist eine Zonula occludens hier nicht zu erwarten. „Gap junctions“, die an den Zellen gleicher Herkunft (Ligamentum spirale) zahlreich vorkommen (Iurato et al., 1974), waren in unseren Präparaten hier nicht sichtbar.

## DISKUSSION

Entsprechend den Beobachtungen an Dünnschnitten zeigen unsere Untersuchungen an Abdrücken gefriergebrochener Präparate der Reissner-Membran, daß endolymphwärts der

Spalt zwischen den Epithelzellen durch Zonulae occludentes abgeschlossen wird.

Die Zonulae occludentes können nach physikochemischen und morphologischen Kriterien in „leaky“ und „tight“ unterteilt werden. Verbände von Epithelzellen mit undurchlässigen („tight“) Zonulae occludentes weisen nach Frömter & Diamond (1972) u. a. einen transepithelialen elektrischen Widerstand von 365 bis 2 000 Ohm  $\text{cm}^2$  auf, ihr Ruhepotential beträgt 30 bis 100 mV. Bei Claude & Goodenough (1973) werden in Abdrücken gefriergebrochener Epithelien mit einem transepithelialen elektrischen Widerstand von 300 bis 600 Ohm  $\text{cm}^2$  die Zonulae occludentes als aus zwei bis sieben übereinander liegenden Kammen, bzw Tälern, zusammengesetzt beschrieben und als „intermediate to tight“ klassifiziert. Diese Werte werden für die „leaky junctions“ wesentlich niedriger angegeben.

Es stellt sich die Frage, ob die Zonulae occludentes des Epithels der Reissner-Membran zum „tight“ oder zum „leaky“ Typ gehören, d. h. ob sie an der Aufrechterhaltung des hohen  $[K^+]$  Gehaltes der Endolymph und des endocochlearen Potentials beteiligt sind. Der transepitheliale elektrische Widerstand dieser Membran beträgt 368 Ohm  $\text{cm}^2$  (Meerschweinchen, Johnstone et al., 1966), das Ruhepotential 30 bis 60 mV (Meerschweinchen, v. Ilberg & Imamura, 1966), die Zahl der Kamme bzw Taler der Zonula occludens nach unseren Untersuchungen beim Chinchilla meistens sechs oder mehr, aber stellenweise auch zwei.

Die Barriere zwischen Endolymph und Perilymphe an der Reissner Membran kann deshalb unter Heranziehung der oben angegebenen Werte für Meerschweinchen und Chinchilla dem Typ „tight“ nach Frömter & Diamond (1972) und „intermediate to tight“ nach Claude & Goodenough (1973) zugeordnet werden.

Die Zonulae occludentes der Reissner-Membran ähneln morphologisch denen der Marginalzellen der Stria vascularis, dagegen weisen sie wesentlich weniger Kämme bzw Täler auf als z. B. die Zonulae occludentes zwischen den Basalzellen der Stria vascularis (Iurato et al., 1974).

## SUMMARY

The Reissner membrane of the chinchilla inner ear was studied with the freeze-fracture method. Zonulae occludentes, composed of 2 to 8 strands, seal the intercellular space close to the endolymphatic surface. They are morphologically similar to those seen between the marginal cells of the stria vascularis, but much less developed than those between the basal stria cells.

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## AN ELECTRON MICROSCOPIC STUDY OF THE OTOLITHIC MACULAE OF THE LAMPREY (*ENTOSPHEUS JAPONICUS*)

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**Abstract** The sensory epithelium of the otolithic maculae from the inner ear of the lamprey (*Entosphenus japonicus*) was studied under the scanning and transmission electron microscope. Two different types of sensory hair cells were discerned and each had a patterned distribution.

Striated organelles were found in the cytoplasm of both types of sensory cells. These striated organelles exhibited periodic electron-dense and less dense striations of 170 nm, extending from the cuticular plate down to the basal portion of the cell. Generally this organelle was found in profusion in the sensory cells with long hair bundles, but rarely found in the other type of cells.

Phylogenetically, the inner ear of the lamprey occupies a unique position in that it has only two semicircular canals instead of the three canals found in other gnathostomes. Because the lamprey labyrinth is considered to be a prototype of the vertebrate labyrinth, detailed studies of the lamprey have been made using both light microscope and electron microscope (de Burlet Versteegh, 1930, Lowenstein et al 1968, Lowenstein & Thornhill, 1970, Thornhill, 1972). Physiologically, the lamprey labyrinth has been shown to be capable of the whole range of responses characteristically found in the labyrinth of the higher vertebrates (Lowenstein, 1970). These responses were in the form of neural activity recorded during angular accelerations in various planes, including the horizontal plane, and recordings of positional and vibrational responses.

It was suggested that the organs responsible for the perception of vibration in the lamprey were localized in the vertical and dorsal maculae. Subsequent to these physiological findings, Lowenstein described a new type of sensory cell characterized by the presence of a long kinocilium and extremely short stereocilia, located preponderantly in the vertical and dorsal maculae. Perception of vibration in the lamprey was mentioned to be related to the presence of these specialized cells in the labyrinth.

This current study was undertaken in an attempt to further elucidate the different types of sensory cells found on the otolithic maculae of the lamprey (*Entosphenus japonicus*). To accomplish this histological study of the maculae, both a scanning (SEM) and a transmission electron microscope (TEM) were employed.

### MATERIALS AND METHODS

Adult lampreys (*Entosphenus japonicus*) with a cephalocaudal length of between 40 and 50 cm were decapitated and the cartilaginous capsule of the inner ear was removed. A 2% phosphate buffered glutaraldehyde solution (pH 7.3) was gently perfused through an opening made in the upper pole of the capsule. The specimens were then submerged in the glutaraldehyde solution (1 to 2 hours for TEM and 2 to 4 hours for SEM preparation). Post fixation was then applied by soaking the specimens for both TEM and SEM study in a 1% phosphate buffered osmic acid solution for one hour.

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## SUMMARY

The Reissner membrane of the chinchilla inner ear was studied with the freeze-fracture method. Zonulae occludentes, composed of 2 to 8 strands, seal the intercellular space close to the endolymphatic surface. They are morphologically similar to those seen between the marginal cells of the stria vascularis, but much less developed than those between the basal stria cells.

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Fig. 2 Two distinctly different types of sensory hair bundles can be seen. The type A bundles (A) have 30 to 40 stereocilia of graded heights (organ pipe configuration) whereas type B bundles (B) have stereocilia of uniform

lengths about  $1\text{ }\mu\text{m}$  and a long kinocilium. (A) anterior horizontal macula (B) posterior horizontal macula) ( $\times 5600$ )

Fig. 3 A portion of the anterior horizontal macula showing the distribution of type A sensory hair bundles (A) over the right side and in a narrow upper and lower

band of the photograph and the type B sensory hair bundles (B) over the remaining left side of the photograph (posterior) ( $\times 380$ )



Fig. 4 Low power view of the vertical macula. The anterior portion of this macula is populated with the type A sensory hair bundles (A). The posterior aspect of this region tapers into a narrow band (arrow posterior to this point the type B sensory hair bundles (B) are found ( $\times 250$ )).



Fig. 5 A view of the entire posterior horizontal macula showing a narrow, extremely lateral distribution of the type A sensory hair bundles (A). The remainder of the macula is occupied by type II sensory hair bundles ( $\times 350$ )).



Fig. 6 A portion of the vertical macula showing both the type A and the type B sensory hair bundles. The two types of sensory hair bundles are morphologically

oppositely polarized in this picture of the vertical macula (arrows) ( $\times 3660$ )

stereocilia of approximately uniform heights ( $1\text{ }\mu\text{m}$ ). A very long kinocilium was found at one end of this bundle of stereocilia and it was distinctly evident.

There was a noteworthy patterned distribution of these two types of hair cells over the surface of each of the maculae observed (Figs 3–5). The type A cells were found in four distinct areas: the anterior half of the anterior horizontal macula with a thin lateral band running posteriorly to the level of the macula sacculi, over the entire surface of the macula sacculi, on the anterior portion of the vertical macula with a thin tapering band, and over a small portion of the lateral edge of the posterior horizontal macula. The remaining segments of the sensory epithelium of the otolith maculae were populated with type B cells (Fig. 7).

The morphological polarization of the sensory cells over the various maculae was not extensively observed in this study, however, a clear polarization was seen on the vertical macula (Fig. 6).

The inner structures of both the type A cells from the anterior horizontal macula and the type B cells from the posterior horizontal macula were examined under the TEM. Both types of hair cells shared the following characteristics: the cell bodies were cylindrical with an ovoid nucleus in the lower portion of the cell, the sensory cell layer was separated from the basement membrane by a supporting cell layer, the cytoplasm of the sensory cells contained many

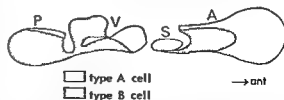


Fig. 7 A schematic drawing illustrating the distribution of the type A and the type B sensory cells over the otolith maculae of the lamprey (dorsal macula not shown). The vertical macula was rotated 90 degrees to illustrate it in the same horizontal plane as the other maculae. A: anterior horizontal macula; S: macula sacculi; V: vertical macula; P: posterior horizontal macula.





*Fig 8* Transmission electron micrograph of type A cells from the anterior portion of the anterior horizontal

macula. Branching striated organelles can be seen in most of the cells (arrows) ( $\times 5100$ )

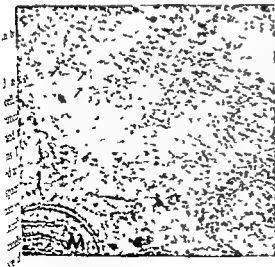
small vesicles and granules and Golgi apparatus and endoplasmic reticulum were also seen

The differences between the type A and type B sensory cells were also found in the cytoplasm of the cells. Type A cells (Fig 8) presented a lower electron density of the cytoplasm due to the reduced amount of granules and vesicles relative to the type B cells (Fig 9). Also structures referred to as striated organelles were found in the cytoplasm of a majority of the type A cells but were absent in most of the type B

cells. These organelles appeared as thin lines which traversed the length of the cell from a point just beneath the cuticular surface passing close to the nucleus and several times in various directions. Throughout its course the organelle appeared to be composed of evenly spaced bands resulting in regular alternations in densities of  $170 \mu\text{m}$ . Higher magnification (Fig 10) suggested that these organelles were composed of an accumulation of th



Fig 9 Transmission electron micrograph of the type B cells (SE) from the posterior horizontal macula. The cytoplasm exhibits a higher electron density than that of the type A cells shown in Fig 8. Striated organelles are not seen in the cytoplasm of these type B cells. SU supporting cell ( $\times 3300$ )



plasmic reticulum. Only a few of the type B cells from the posterior horizontal macula contained this striated organelle. However, when this structure was seen in a type B cell it appeared to be much less developed and it was usually localized in the upper portion of the cell, i.e., the subcuticular or supranuclear region.

The sensory cells were innervated, at their bases, by means of two distinct types of nerve endings (Fig 11). One nerve ending type contained within it many mitochondria and only a few vesicles (Fig 11-1). In the adjacent sensory

Fig 10 Higher magnification photograph of the striated organelle. The organelle seems to be composed of an accumulation of thin tubular structures (arrows). M mitochondria ( $\times 60000$ )



Fig 11 The different types of nerve endings seen at the base of the sensory cell 1 A nerve ending containing many mitochondria (*M*) and only a few vesicles. A round electron-dense body surrounded by layers of vesicles can be seen in the sensory cell body (*SE*). This is assumed

to be a synaptic body accompanying an afferent ending ( $\times 67\,000$ ) 2 A nerve ending containing vesicles. A subsynaptic cistern (arrow) can be seen in the sensory cell body (*SE*) close to the synaptic membrane of the supporting cell (*SU*) ( $\times 30\,000$ )

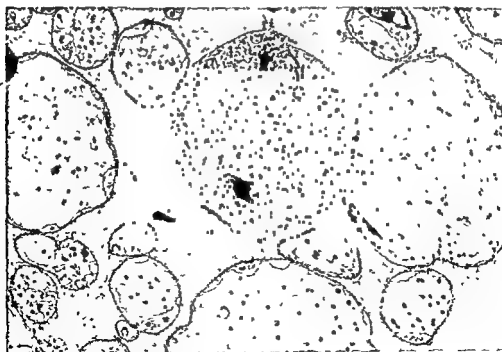


Fig 12 Unmyelinated nerve fibers running beneath the posterior horizontal macula. Marked differences in the

diameters of these fibers can be seen. *G* = ganglion cell body ( $\times 3\,500$ )

1 a homogeneous dense body (synaptic body) s frequently found located near the thickened iaptic membrane and surrounded by layers of iacles. The other type of nerve ending exhibited a ckened membrane on both the nerve ending d on the adjacent sensory cell body. As posed to the first type of nerve ending scribed above, this second type (Fig 11-2) tained many small vesicles (with diameters 60 to 70  $\mu\text{m}$ ), a few larger cored vesicles 0 to 120  $\mu\text{m}$  in diameter) and a few mito- ndria. Also, a sub-synaptic cistern was seen the second type of nerve ending (arrow, g 11-2).

No distinct pattern was discerned relative to innervation of the type A and the type II isory cells by these two different types of nerve dings. Judging from the results from previous estigators regarding sensory endorgan syn- ses, it seems likely that these two types of, ve endings correspond to the afferent (Fig -1) and efferent (Fig 11-2) endings.

The nerve fibers which course beneath the terior and the posterior horizontal maculae re found to be all unmyelinated. However, re was wide variation in the diameters of these ers (Fig 12).

## COMMENTS

the older literature (de Burlet & Versteegh, 30) the nomenclature of the otolithic maculae the lamprey was based on the homology of gnathostomes. However, Lowenstein et al 68) recently advocated a revision of this nomenclature based upon a simpler topo- phical view of the lamprey labyrinth. In this rent report, Lowenstein's revision has been opted with the exception of the macula culi for which the older classical term was d. In the present study, the polarization of the sory cell cilia was not extensively observed l thus further ideas regarding classification l nomenclature have not been tendered. ensory cells bearing uniformly short stereo- and a long kinocilium had previously been cussed by Lowenstein et al (1968). These

cells were found predominantly on the vertical and dorsal maculae, the maculae from which the responses to vibration were electrophysio- logically recorded by the same author (L6wen- stein, 1970). However, in the current study, this particular type of sensory cell (type B) was to be confined not only to the vertical and dorsal maculae. They were also distributed rather profusely over the posterior segment of the macula anterior horizontal and also over most of the posterior horizontal macula. This discrepancy is most likely based on the fact that L6wenstein et al (1968) described the otolithic maculae of the *Lampetra fluviatilis* whereas this present study examined the maculae of the *Entosphenus japonicus*. Regardless, the results of this current study suggest that the perception of vibration in the lamprey cannot be solely attributed to the hair cells with short stereocilia and a long kinocilium (the type II cells).

The striated organelle found within the labyrinthine sensory cells of the ammocoete larva of the lamprey was first described by L6wenstein & Osborne (1964). However, these authors did not discuss the major internal differences in structure amongst the cells which they examined. In the current study it could be seen that the development of the striated organelle was more complete in the type A cell relative to the type II cell.

Similar striated structures have been discerned in sensory cells taken from pathological otolithic maculae, i.e., from patients with Meniere's disease (Friedmann et al, 1963) and from animals treated with ototoxic drugs (Jahnke 1969). These intracellular striated structures have also been found in non labyrinthine cells taken from non pathological speci- mens, i.e., from the tympanic muscles of the cat (Hirayama & Daly, 1974), in the extra- ocular muscles of humans (Mukuno, 1966), in the neurons of the geniculate nucleus of the cat (Smith et al, 1964), in the lateral geniculate body neurons of the cat (Morales et al, 1964) and at the receptor synapses of the guinea pig retina (Mountford, 1964).

Striated organelles found in sensory cells

from pathological labyrinthine sensory epithelium (i.e., Menière's disease or experimental ototoxicity) show a very thin transverse line located between each of the regularly spaced electron-dense bands. Also, in these pathological cells, the striated organelle is found only in the immediate subcuticular area of the cell. Striated organelles in normal sensory cell, as reported in the study, did not exhibit this intermediate transverse line. Higher magnification views of the electron-dense bands on normal sensory cell organelles indicated that these bands were actually composed of closely spaced, longitudinally oriented, tubular structures probably consisting of differentiated endoplasmic reticulum. Although a stimulus conducting role or even an intracellular support role could be attributed to these striated organelles, no particular conclusion could be offered concerning their function. Thus the differential distribution of these organelles in the type A and the type B cells sheds little light on their possible functional differences.

It is felt that the observation of two different types of sensory cells in the otolithic maculae of the lamprey is rather noteworthy. Although many differences regarding the morphology of the nerve endings and the general shape of the sensory cells from the mammalian otolithic maculae have been published, there has been little discussion regarding distinct differences between the sensory hairs or between the specific intracellular organelles. In a few SEM studies of the otolithic maculae of fish and amphibians (Arenberg & Rauchbach, 1973; Lewis & Li, 1973), differences in the lengths of the sensory hairs on cells from different maculae were discussed. However, these reports did not show a clear-cut dichotomy between the hair cell bundle types or between the presence of striated organelles in the sensory cell bodies as found in the lamprey. It seems likely that further physiological studies of vibration and equilibrium perception will elucidate functional differences underlying the morphological differences between the two types of sensory cells from the otolithic maculae of the lamprey.

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## ZUSAMMENFASSUNG

Die morphologischen Einzelheiten des Sinnes vom Lampreteninnenohr wurden im Elektronenmikroskop und Rasterelektronenmikroskop untersucht. Arten von Haarzellen mit verschiedenen Sinneshaaren wurden in der Macula gefunden. Der eine Zelltyp hatte 20-30 kurze Stereozilien und ein langes Kink an der Oberfläche. Der andere Typ hatte noch Stereozilien mit stufenweise erhöhter Haarlänge. Die ersten Teile der Zellen von zwei verschiedenen Zelltypen zeigten sogenannte „striated organelle“ in der Plasmamembran. Im allgemeinen wurden diese Organelle häufig in den Sinneszellen mit den langen Haaren gefunden, dagegen im anderen Zellentyp kaum gefunden. Verschiedenen Haarzellen wiesen eine bestimmten Teilung in jeder Macula des Lampreteninnenohrs.

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## METHODOLOGIC OBSERVATIONS ON TYMPANOMETRY WITH REGARD TO THE PROBE TONE FREQUENCY

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**Abstract** Changes in the tympanogram shape in the frequency range from 200 to 2000 Hz have been investigated. The tympanogram undergoes, as the probe tone frequency rises, characteristic morphologic modifications which are consistently observed both in normals and in some middle ear disorders. The only exception to this regular behavior is the pathological picture in which the structure or the dynamic of the tympano-ossicular system is remarkably anomalous (cholesteatoma and otitis media) where there are flat tympanograms. It is suggested that the frequency interval at which one of the characteristic tympanometric configurations appears is the element capable of furnishing a diagnostic tool for differentiating pathophysiologic conditions of the middle ear.

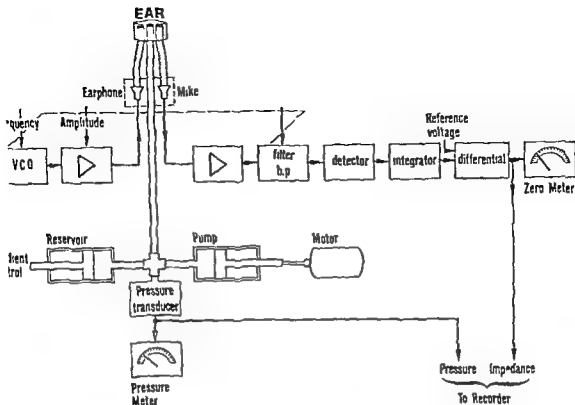
It is known from many investigations that a difference in the static pressure on the two sides of the tympanic membrane change the acoustic properties (functions) of the middle ear and consequently produce recordable changes in the ear's acoustic impedance (Thomsen, 1955, Anderson et al., 1956, Therkildsen & Thomsen, 1959, Møller, 1965).

On the basis of the above-mentioned experimental findings, a clinical method known as tympanometry has emerged for assessing the ear drum mobility, the status of the ossicular chain, the middle ear pressure and the Eustachian tube function (Brooks, 1968, 1969, Lidén et al., 1970, Jerger, 1970, Olivier, 1971, Bel et al., 1972, Marquet et al., 1973, Van den Eeckhout et al., 1973). Tympanometry consists, in short, in measuring the ear's acoustic impedance as a function of an externally applied air pressure in the ear canal. While the validity of tympanometry in assessing and discriminating among

various middle ear pathologic conditions has been demonstrated by several of these papers, there is still controversy as to what constitutes the most effective probe tone frequency. Recent literature reports two main opposing opinions and experience.

Alberti & Jerger (1974), in a comparative analysis of the tympanograms obtained with probe tone frequencies of 220 and 800 Hz in various types of middle ear disorders, assert the occurrence of a W pattern observable at 220 Hz, seems most closely related to disease of the tympanic membrane but, unfortunately, the W pattern may also be a normal variant. Therefore the authors concluded that "there does appear to be substantial clinical value in plotting tympanograms at probe tone frequencies higher than 220 Hz". Lidén et al. (1974), on the contrary, stress that in order to gain the maximum valuable information for the differential diagnosis of conductive impairments, the tympanometric investigation should be carried out with a probe tone frequency of 800 Hz, a frequency within the resonance frequency range of the human middle ear.

In a preliminary report, Colletti (1974) discussed the methodologic reasons for using a probe tone of variable frequencies (200 to 2000 Hz). The present investigation concerns the relation between the pattern of the tympanogram and the frequency of the probe tone, the alteration of the shape of the tympanogram as a function of various middle ear pathologic



1 Block diagram of the equipment used in multi-frequency tympanometry

o discussed The relation between the tympanogram and the intensity of the probe tone as well as the static pressure, to be imposed in a ear canal, will be reported in a subsequent publication (Colletti, in preparation)

## METHOD

■ basic equipment that we now routinely use for tympanometry is shown in the block diagram of Fig 1 The reader is referred to a previous paper for technical details (Colletti, 1974) The ear canal and the tympanic membrane of the patients were examined before the initiation of tympanometry Tympanograms were measured first at 200 Hz and then repeated with 200 Hz increments up to 2 000 Hz the highest frequency tested When a new tympanometric shape appeared in the investigation the frequency resolution was increased, and steps of 20 Hz were used Normally the pressure was kept between

-200 and +200 mmH<sub>2</sub>O If the impedance/pressure function revealed a flat or flat-descending slope, the range of pressure values was increased to  $\pm 400$  mmH<sub>2</sub>O The rate of change of the pressure gradient was kept at 10 mmH<sub>2</sub>O/sec and the sound level was below the individual threshold of the stapedius reflex The output signals from the pressure transducer and the electroacoustic bridge were recorded on a two channel recorder (Electronystagmograph Mod Galileo) The air pressure in the ear canal, and the corresponding impedance value could thus be observed simultaneously

## SUBJECTS

Two groups of subjects will be reported in the present study The first group consisted of normal hearing subjects and included twenty ears The subjects selected were medical students Ears with progressive or actual pathology were



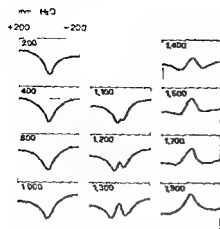


Fig. 2 Tympanograms at several probe tone frequencies obtained in a normal hearing subject with normal tympanic membrane for decreasing pressure values (from +200 to -200 mmH<sub>2</sub>O) at 10 mmH<sub>2</sub>O/sec pressure gradient. The tympanograms have been subdivided in three groups according to the morphologic similarities of the impedance/pressure functions versus frequency.

excluded from our test procedures. Pure tone audiograms and stapedius and tensor reflex examinations furnished evidence of normal hearing levels and normal middle ear status.

The second group comprised a selection of various middle ear pathophysiologic conditions and included twenty pure otosclerotic ears, i.e. not complicated by progressive flogistic epibulbar, eleven successfully stapedectomized ears, eleven otosclerotic fixation of the stapedio-ovalar joint, six ossicular discontinuities, three post-myringoplastic ears, two ears with attic cholesteatoma with intact ear drum and finally ten cases of serous otitis media in the acute phase of the disease process.

## RESULTS

In patients with normal middle ear function, the shape of the tympanogram varies in a systematic way with the probe tone frequency. The depth of the simple tympanograms, however, shows a great individual variability. All subjects showed these characteristic shapes of the tympanograms which corresponded to three different ranges of probe tone frequencies. Fig. 2 is an example of typical tympanograms in a subject with normal hearing and normal ear drum. The

three patterns are clearly seen and characterized as follows:

- (1) For low frequencies the tympanograms are V-shaped, thus showing a single maximum with symmetrical tails.
- (2) With mid-range frequencies (usually 1100–1300 Hz) the tympanogram shows a gradual evolution to a W shape.
- (3) At higher frequencies (above 1500 Hz) the tympanogram assumes an inverted V shape with a single maximum and symmetrical tails.

The primary concern of the investigation was to determine the frequency range of each pattern. Therefore the absolute impedance will not be reported. In most of the pathologies (otitis media, post-stapedectomy, ossicular disarticulation and post-myringoplasty) the three characteristic tympanogram patterns are found. The transition from one pattern to another, however, occurs at a different probe tone frequency range, as may be observed in Fig. 3. If we simply note at what frequency the W shape occurs, the following relationship can be observed:

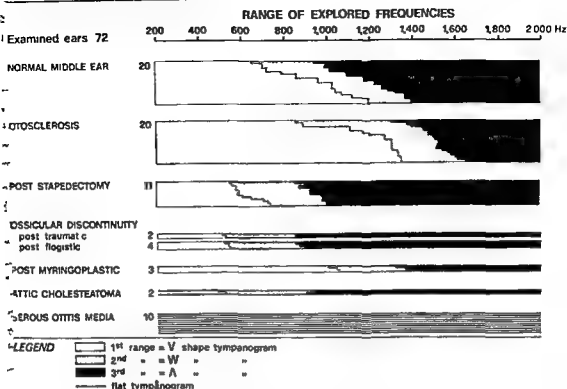
- (1) Normal subjects, the W pattern is observed at frequencies between 650 and 1000 Hz.
- (2) Otosclerotic ears, the emergence of the W pattern occurs at higher frequencies, i.e. above 1650 Hz.
- (3) Post-stapedectomized and ossicular discontinuity present a W configuration in the probe tone frequency range from 500 to 1000 Hz. The transition of the single ranges in this group shows a very limited overlapping with the normal range.
- (4) The post-myringoplastics' frequency range is within the lower and upper boundaries of the normals, i.e. from 1000 to 1400 Hz.

An example of the transition from V to inverted V pattern is given, in detail, in Fig. 4.

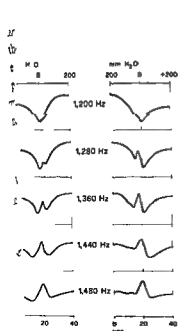
Two exceptions to this frequency-dependent tympanometric pattern are seen in serous otitis media and cholesteatoma.

The cholesteatoma cases manifested a flat tympanogram for low frequencies which evolved to a W pattern above 500 Hz and finally inverted at 900 Hz. An example is shown in Fig. 5.

# PLURIFREQUENCY TYMPANOMETRIC INVESTIGATION



## Plurifrequency tympanometric investigation on 72



The serous otitis media, on the other hand, displayed a flat tympanogram at all frequencies tested even with maximum pressure excursions of  $\pm 400$  mmH<sub>2</sub>O

## DISCUSSION

The present investigation suggests that the single tympanometric configuration observed by different researchers at 220, 660 and 800 Hz (quoted in the introduction) are simply typical, and single

**Fig 4** Dynamic of the tympanogram shape inversion. The frequency range from 1 200 to 1 480 Hz is reported both for decreasing (left side) and increasing (right side) air pressure values. The pressure gradient is kept constant at 10 mmH<sub>2</sub>O/sec. Significant morphologic differences are appreciable as a function of the pressure direction. The tympanogram configuration is reversed in respect to the horizontal axis in an interval of approximately 300 Hz.

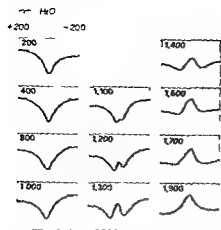


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(3) At higher frequencies (above 1300 Hz) the tympanogram assumes an inverted V shape with a single maximum and symmetrical

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membrane produces a flat tympanogram at low frequency range, which evolved to smooth W and later inverted V at higher frequencies (actual findings from a patient are reported in Fig 5). This situation could not have been discriminated in other conditions such as serous otitis media or massive tympanosclerosis on the basis of the tympanogram if the analysis had been limited to 220 Hz. In fact, only over 500 Hz did the tympanometric configuration assume the second stage and, very preliminarily, the third range as a possible result of an increase of the mass component of the ossicular system in concurrence with a reduction of the tympanic membrane elasticity. Unfortunately, there are only two cases of cholesteatoma and generalizations must be tempered by the smallness of the sample, but results of these 2 patients were reliable to be consistent across both cases.

For future studies, it would be of interest to analyse whether a relationship exists between the shifting of the W shape towards high frequencies, and the degree of stapes fixation. Particularly, it would be useful to study a large group of post-stapedectomized ears, using tympanogram configuration in order to determine the effect of the different surgical techniques and the thesis generally used (area of the exposed window, mass and length of the prosthesis, mobility of its attachment to the long process of the incus, and so on). Studies in progress indicate that pre- and post-operative comparative tympanometric examinations might well follow the evolution of a post-stapedectomized to be followed on an individual basis.

## RESUME

Les modifications du tympanogramme dans l'intervalle de fréquence de 200 à 2000 Hz sont étudées. Le tympanogramme, en augmentant la fréquence d'exploration en continuité, présente de caractéristiques modifications de sa forme, qui sont constamment observables dans l'oreille normale aussi que dans les différentes maladies de l'oreille moyenne. Une atypie de ce comportement est représentée par certaines conditions pathologiques auxquelles la dynamique ou bien la structure du système tympano-ossiculaire est sensiblement altérée. L'intervalle de fréquence à laquelle les différents tympanogrammes sont incluses représentent, dans notre

expérience, l'élément capable de suggérer des informations diagnostiques dans les différentes conditions physiopathologiques de l'oreille moyenne.

## ZUSAMMENFASSUNG

Im Frequenzbereich von 200 bis 2000 Hz sind die Änderungen der Tympanogramme von Normalhörenden und Patienten mit unterschiedlichen Mittelohrkrankheiten untersucht worden. Bei wachsender Frequenz des Testtons zeigt das Tympanogramm sowohl bei Normalhörenden als auch bei einer Reihe von Mittelohrkrankungen durchgehend bestimmte charakteristische Veränderungen. Die einzige Ausnahme in diesem regelmässigen Schema sind die Fälle, bei denen im pathologischen Bild die Struktur oder die Dynamik des Tympano-ossikular-Systems deutlich anomal ist, nämlich bei Cholesteatomen und bei Otitis media; hier weist das Tympanogramm keine Änderung gegenüber der Frequenz auf. Aus den Untersuchungen lässt sich entnehmen, dass der Frequenzbereich der eine der charakteristischen Formen des Tympanogramms erkennen lässt, als solcher ein diagnostisches Indizium für die Differenzierung physiopathologischer Veränderungen im Mittelohr ist.

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## MODIFICATIONS OF THE STAPEDIUS MUSCLE REFLEX UNDER SPONTANEOUS AND EXPERIMENTAL BRAIN-STEM IMPAIRMENT

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**Abstract.** An oscillographic plus a graphic method of recording of the stapedius muscle reflex has revealed various modifications in the latency, threshold, and rate of development of the reflex in cases of brain-stem impairment. Many subjects have been examined who were affected by nucleo-reticular vestibular syndrome, vestibular insufficiency, disseminated sclerosis, tumours, and a comparison has also been made in normal subjects and in the same subjects during a temporary impairment of the brain-stem structures following barbiturate administration. The greatest semeiological value is attributed to the modifications in the shape of the reflex.

As already been observed that the relative impedance studies of the middle-ear muscle reflexes, originally destined to investigate the various pathological conditions of the middle-ear effector structures, have subsequently proved increasing value also in studying the functional conditions of their supporting nervous system. Thus, a lowering of the physiological threshold gap of the stapedius reflex has been demonstrated in cases of end-organ deafness, at the beginning of the afferent arch, thus giving an objective demonstration of the impairment phenomenon (Metz, 1951; Liden, 1961). Subsequently, an abnormally rapid decay of the reflex contraction, due to an impairment of VIII nerve fibres, has been demonstrated, this can be a very precocious sign of acoustic neuroma (Anderson et al., 1970).

In the same way, an interruption of the efferent arch of the stapedius reflex reveals the site of damage along the VII nerve.

Very few reports have so far appeared regarding modifications of the stapedius reflex due to damage of the intermediate portion of the nervous arch, between the cochlear and the facial nuclei.

In a previous note (Bosatra & Russolo, 1970) we have described the cupula-like pattern of the stapedius reflex as traced by a graphic recorder in patients affected by a moderate, temporary impairment of the brain-stem structures (acute nucleo-reticular vestibular syndrome) due to exogenous or endogenous intoxication. In these cases the hearing at threshold and suprathreshold levels was normal and the lesion was characterized by spontaneous and instrumental signs of vestibular impairment.

In these same cases also the latency of the reflex and its threshold were increased (Bosatra & Russolo, 1973; Bosatra et al., 1973).

In this paper we will refer to the modifications of the various parameters of the stapedius reflex as observed in various types of pathological conditions and during experimental temporary impairment of the function of the central nervous system, with predominant effect at the level of the brain-stem structures, obtained by barbiturate administration.

### MATERIAL

The following subjects have been examined

(1) 25 healthy individuals of normal weight and body constitution, aged 15-35, with normal

hearing (10–15 dB SPL) and normal tympanometry

(2) The same 25 individuals, during the temporary impairment of the tonic activity of the central nervous system, especially evident at the brain stem level, obtained by barbiturate administration (sodium pentobarbital 3–4 mg/kg, per os)

(3) 15 patients affected by acute nucleoreticular vestibular syndrome, showing normal hearing and tympanometric tracings but complaining of vertigo, with spontaneous nystagmus of varying intensity and direction, hyporeflexivity and saccadic ENG tracings

(4) 10 patients effected by cerebral vascular insufficiency, with clinical, ENG and EEG signs of prominent lesion at brain stem level, with slight presbycusis (hearing threshold from 10 to 50 dB SPL, with normal tympanometry) and complaining of attacks of vertigo and showing spontaneous nystagmus of varying intensity

(5) 15 patients affected by disseminated sclerosis with normal hearing (10–15 dB SPL) but with spontaneous nystagmus, hyperreflexivity, with irregular ENG tracings and other neurological signs of the disease

(6) 2 patients affected by brain stem tumour (astrocytoma). Both patients showed slight deafness with symmetrical hearing threshold at 30–40 dB SPL with spontaneous nystagmus and ENG tracings of central type

(7) 1 patient affected by unilateral, extra-mesencephalic angle tumour, with well preserved hearing (30 dB SPL), bilateral spontaneous nystagmus and homolateral brain-stem involvement

(8) 1 patient affected by syringobulbia

## METHODS

The stapedius muscle reflex was studied by using a Madsen ZO 70 or Madsen ZO 72 Impedance Meter connected to a graphic recorder and to a dual beam storage oscilloscope

The subjects were examined in a silent room, sitting or lying on a stretcher, with closed eyes, avoiding head movements. The reflex was

elicited by pure tones of 4 000–2 000–1 000 Hz presented through an earphone in the described order

The reflex threshold was detected through a graphic recorder by using stimuli of 1 s duration, 10  $\mu$ sec rise and fall time, at 10 steps of 1 dB

The reflex latency was measured at 10 over-threshold and at 120 dB SPL, by using a dual beam storage oscilloscope. The latency of the reflex was evaluated by averaging responses elicited by tones of 500 msec duration, 10  $\mu$ sec rise and fall time, identical for frequency and intensity. The stimulus repetition was 1 per second, every 25–30 sec. The successive oscillographic tracings of the reflex were memorized in the first channel and the series of the tones was memorized and superimposed on the second channel

The reflex shape has been graphically recorded at 10 dB overthreshold with the same tones, of 10 sec duration. The onset of the reflex therefore has been examined both on the oscilloscope and the graphic recorder

## APPARATUS

A function generator Unaohm EM 95 A was used for pure tones, the duration, the rise and fall time, the intensity of the test tones were controlled by an electronic switch (G Stadler 1287), an attenuator at 1 dB (Grason-Stadler 1292), an amplifier (G

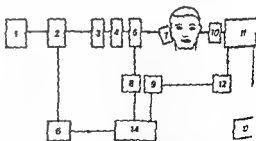
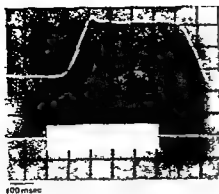
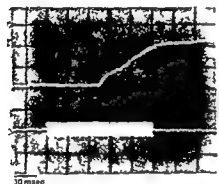


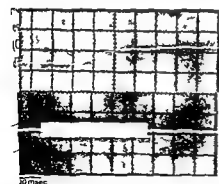
Fig. 1 Instrumentation block diagram. 1 Function generator, 2 Electronic switch, 3 1 dB steps attenuator, 4 Amplifier, 5 5 dB steps attenuator, 6 Transducer, 7 Earphone, 8 9 Differential amplifiers, 10 Subject, 11 Impedance meter, 12 Preamplifier, 13 Graphic recorder, 14 Oscilloscope



2 Oscilloscope tracing in normal subject. Note longer latency and sharp rising of the reflex A reflex stimulus (1000 Hz)



3 Oscilloscope tracing in the same subject as Fig 2. Note longer latency and slow step-like rise A reflex B stimulus (1000 Hz)



4 Oscilloscope tracing of stapedius reflex in acute otorecticular vestibular lesion. Note longer latency slow step-like rise A reflex B stimulus (1000 Hz)

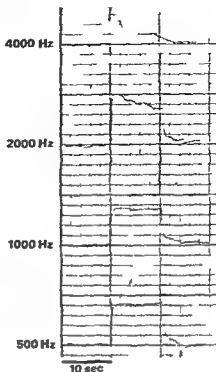


Fig 5 Graphic record of stapedius reflex in normal subject

taneously by a series of timers (Grason Stadler 1216 A)

The tones were presented through an earphone (Fujiki DR 59). The responses were studied by a Madsen ZO 70 or ZO 72 Impedance Meter connected to a graphic recorder Goerz Miniator RE 501 and to a dual beam storage oscilloscope Tektronix 5103 N/D 13 (Fig 1)

## RESULTS

### Reflex threshold

In the normal subjects the threshold at the four frequencies (4000-2000-1000-500 Hz) ranged from 80 to 90 dB SPL. Under pathological conditions the values altered as follows:

Subjects under the action of the barbiturate 90 to 95 dB SPL

Patients affected by acute nucleoreticular vestibular syndrome (nrvs) 90 to 95 dB SPL

Patients affected by cerebral vascular insufficiency 80 to 120 dB SPL

Patients affected by disseminated sclerosis 80

Stadler 1288) an attenuator at 5 dB steps (Grason Stadler 1293)

The order of tone presentation and the trigger of the oscilloscope were controlled simul-



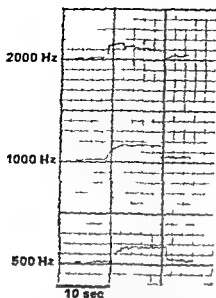


Fig 6 Graphic record of stapled ear reflex in same normal subject of Fig. 5 during barbiturate action. Note cupula-like pattern during the rising phase of the reflex and reduced amplitude.

Subjects under barbiturate action from 390 msec

Patients affected by acute n.r.s. from 360 msec

Patients affected by brain stem impairment to disseminated sclerosis or vascular encephalopathy and also patient affected by a pontocerebellar angle tumour from 400 msec

### Shape of the reflex tracings

The formal aspect of the stmr elicited at 110 dB overthreshold has been examined with an oscilloscope and a graphic recorder (see Fig. 2).

In normal subjects the graphic or oscillographic tracing was rising at the onset sharply (Figs 2-5).

In the same subject during the barbiturate effect (Fig. 3) and in several of the patients affected by brain stem impairment, and by a pontocerebellar angle tumour (Fig. 4) disseminated

120 dB SPL. In 4 subjects belonging to this group examined during an acute phase of the disease the reflex was absent. In 3 out of the same 4 patients tested after some months during a phase of clinical recovery the reflex threshold was obtained within normal values at 1000 and 500 Hz and at 110 dB SPL at 4000 and 2000 Hz.

Patients affected by brain stem tumour: bilateral absence of the reflex.

Patient affected by unilateral pontocerebellar angle tumour: reflex present at 110 dB SPL for 1000 and 500 Hz only, with strong decay homolateral to the lesion (where the earphone was applied). Reflex present at 100 dB SPL but peculiar cupula-like pattern on the contralateral side.

Patient affected by syringobulbia: reflex bilaterally absent.

### Latency

In normal subjects the latency of the stmr examined with the above mentioned methods ranged from 40 to 180 msec.

Under pathological conditions the latency was always lengthened as follows:

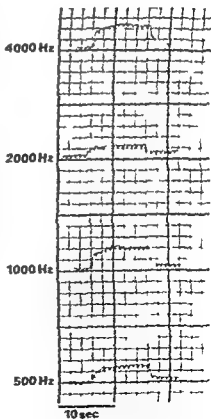


Fig 7 Graphic record in acute nucleo-reticular lesion. Note slow onset of the reflex.

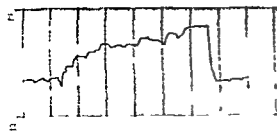
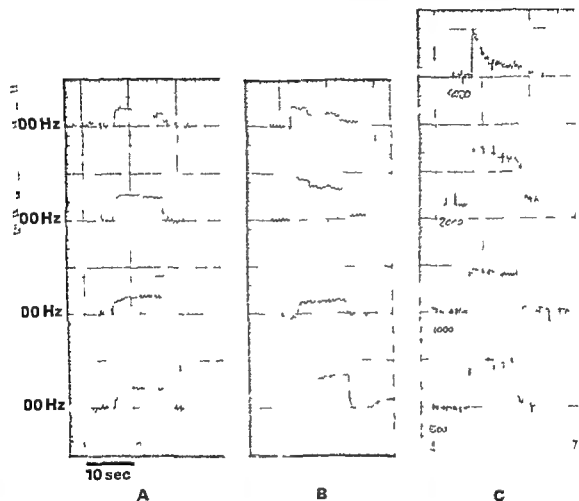


Fig 8 Enlarged graphic record of stapedius reflex in nucleorebular vestibular lesion 1000 Hz, 10 dB over threshold 10 sec duration



Frequencies	St m r	Latency	St m r	Latency	St m r	Latency
	threshold	at 120 dB	threshold	at 120 dB	threshold	at 120 dB
0 Hz	100 dB	190 msec	95 dB	190 msec	90 dB	170 msec
0 Hz	100 dB	170 msec	95 dB	160 msec	90 dB	140 msec
0 Hz	85 dB	160 msec	85 dB	150 msec	85 dB	120 msec
0 Hz	95 dB	160 msec	95 dB	150 msec	95 dB	120 msec

9 Modifications of various parameters of stapedius reflex during disseminated sclerosis (A) Acute phase (B) During recovery (after 10 days) (C) Complete recovery (after 3 months)

sclerosis, etc., the oscillographic tracing was rising slowly with peculiar saccadic steps

With the graphic recorder the same phenomenon appeared as a cupula like pattern of the rising phase

This peculiar shape of the tracings was regularly present in the barbiturate group (Fig 6), quite regularly present in the patients affected by acute n r v s (Figs 7, 8), less regularly present in the cases of vascular insufficiency or disseminated sclerosis

In these same cases the amplitude of the responses was often reduced, but in the vascular patients it was sometime increased. However, there was no tendency towards a fast decay

It can be added that in all pathological conditions the tracings appeared to be less stable and regular than in the normal ones

## CONCLUSION

By applying an oscillographic plus a graphic method of recording of the stapedius reflex we have observed peculiar modifications in cases of pathological or experimental impairment of the central nervous system and especially at the level of the brain stem

Thus the modifications of the reflex can be attributed to an impairment of the central portion of its nervous reflex arch. This concerns the threshold, the latency and the shape and development of the reflex contraction especially during the rising phase. This latter phenomenon is the more easily appreciable and therefore we believe it bears the greatest semeiological significance

It can be suggested that this peculiar shape represents a sort of desynchronization of the conduction of the stimulus at the brain stem nucleo-reticular level

In some cases of vascular insufficiency, disseminated sclerosis, tumours and syringobulbia, we have observed an absence of the reflex. This has been already described in a few other cases by Greisen & Rasmussen (1970)

The absence may also be of some value, especially when a bilateral comparison can be made, or during the course of the disease as we have observed in some of our cases of disseminated sclerosis (see Fig 9)

## ZUSAMMENFASSUNG

Durch die Anwendung einer Methode mit Oscillographen und graphischen Geräten zur Reflexregistrierung des Stapedius konnten bei einer Reizung des encephalicus Veränderungen der Latenz, der Amplitude und der Reflexformen registriert werden. Es wurden 10 Patienten untersucht, die vestibuläre, retikuläre Syndrome aufwiesen sowie vaskuläre, Sklerose Tumoren usw. Weiterhin wurden 10 gleich zwischen Patienten gezogen worden, bei denen zuerst keine Behandlung mit Barbituraten und eine derartige Behandlung durchgeführt wurde. Der grösste Wert des Experiments besteht in der Feststellung der Veränderung der Art des Reflexes

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## SISI TEST AND ADAPTATION

### II Subjects with Conductive Hearing Defects

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The effect of adaptation of the ear on the SISI was studied at 500 Hz and 2 000 Hz in 43 patients with chronic otitis and in 20 patients with otosclerosis. Mean adaptation was slightly over 10 dB, except in the otosclerosis group at 2 000 Hz, for which the mean was nearly 20 dB after continuous stimulation at 20 dB SL for 3 min. The mean SISI values ranged from -10 to 31% in the preadaptation tests and from 9 to 32% in the postadaptation tests. The scatter of the preadaptation SISI scores was wide, making statistical treatment of the results difficult. In the total material 22% of the values at 2 000 Hz were positive according to Jerger's classification, and 32% were questionable. Adaptation slightly reduced the proportion of positive values, but there were changes towards both higher and lower levels in the cases of chronic otitis, while in all the otosclerosis changes were diminutions.

The effect of adaptation on the SISI test in 26 subjects with normal hearing has been reported previously (Rahko, 1971). After the conventional hearing test had been administered, the test ear was adapted for 3 min with a sustained 20 dB SL tone. SISI scores of between -10 and 6 000 Hz were obtained immediately before and after adaptation. The statistical dispersion of both pre- and postadaptation values was found to be large. Adaptation caused no significant change in either direction in the SISI scores, the proportion of which increased and some decreased. About 15% fell within the positive group, according to the classification of Jerger (1961). Initially, Jerger (1959) found that the SISI scores in his material of 21 subjects with conductive hearing defects, of 5 to 60 dB at 1 000 Hz and of 15 to 60 dB at 4 000 Hz, ranged from

0% to 15% at both frequencies. No diagnostic classification of the patients was given. In a later study (1961), he reported negative SISI scores in 15 cases, questionable ones in 4 and positive ones in 2. The SISI scores were now classified as follows: 0-15% negative, 20-55% questionable and >60% positive. The recruitment phenomenon, measured using Fowler's alternate binaural loudness balance (ABLB) was negative in all cases. Similar low SISI scores were also obtained by Young & Harbert (1967) in cases of conductive hearing defects.

The suprathreshold adaptation tests (Palva 1955, 1964, Kärjä, 1968, Palva & Kärjä, 1969) have shown that even normal ears develop a marked adaptation at 20 dB sensation level (SL). No tests have been performed on ears with conductive hearing defects at this level. At 60 dB SL and 2 000 Hz Kärjä (1970) found adaptation to be at its maximum, of the order of 30 dB, in cases of otosclerosis and of chronic otitis media.

In the SISI test the 20 dB SL tone lasts 1 min 40 sec, which permits considerable adaptation.

The present study was carried out in order to investigate the SISI values in conductive hearing defects, and to test whether adaptation affects these values.

### MATERIAL AND METHODS

The series consisted of 63 outpatients, 38 women and 25 men, mean age 32 years, at the Oulu University Department of Otolaryngology.

This study was aided with a grant from the State Medical Research Council.

Table I *Average audiological findings of the conductive material in various groups*

Frequency (Hz)	Pure tone hearing thresholds (dB, ISO)								3 min tone decay test (dB)				Recruitment A&L when 100 dB SL Number of cases amount of recruitment			
	Air conduction				Bone conduction											
	AE		CE		AE		CE		AE		CE					
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	10-25 dB	or more		
Chronic otitis																
500	41.1	10.0	8.0	8.5	8.0	8.6			3.2	4.0	2.7	2.6	33	10	11	
2000	34.7	10.7	6.5	5.8	5.7	6.4	5.0	6.1	8.0	8.4	5.8	6.3	29	14	0	
Otoscle rosis																
500	43.0	10.7	9.5	6.3	10.8	6.5	10.0	3.5	5.0	3.0	2.0	2.6	12	8	0	
2000	36.8	11.4	8.0	6.6	11.3	9.2	5.0	3.5	5.8	6.9	5.3	6.0	15	3	0	
Total material																
500	41.7	10.2	8.6	7.8	7.9	5.6	10.0	3.2	4.0	3.6	2.4	2.6	45	18	0	
2000	34.8	11.7	6.9	6.1	7.3	7.8	4.5	4.7	7.3	8.0	5.5	6.0	44	19	0	

They all had unilateral conductive hearing defects, with normal hearing in the contralateral ear. 44 had chronic otitis media and 20 had otosclerosis.

Only those patients for whom the difference between the hearing thresholds did not exceed 50 dB were included in the analysis of supra-threshold adaptation. In this situation, with one normal ear, interaural over-hearing does not affect the examination of supra-threshold adaptation when insert receivers are used (Palva & Palva, 1962). Supra-threshold adaptation was measured at 500 Hz in 35 patients with chronic otitis media, at 2000 Hz in 41 such patients, and at both frequencies in all 20 cases of otosclerosis.

The following basic audiological tests were performed on every patient. A pure tone audiogram was taken using the ascending-descending method with air and bone conduction thresholds. The equipment used was a Madsen OB 60 audiometer with TDH 39 earphones calibrated according to the ISO standard. The bone conduction thresholds of the control ear were not always ascertained, provided that the air conduction threshold was normal. Speech audiometry was carried

out using the Finnish language test of (1952), measuring the speech reception threshold (SRT) and also the speech discrimination at a level of 30 dB above the SRT. The equipment used for this test comprised a Madsen SU 20 speech unit connected with a Madsen OB 60 audiometer.

A 3 min threshold adaptation test was included by increasing the adapting tone in steps in the Madsen OB 60 audiometer test. This test was not performed on all patients at 500 Hz, unless the 2000 Hz value had indicated adaptation. Loudness recruitment was measured according to the Fowler ABLB technique. In suitable cases filtered speech test after (1965) was also performed. However, due to large threshold differences, the filtration could be carried out on only 7 patients with chronic otitis and 2 with otosclerosis.

Further details of the equipment and modifications were given in an earlier paper (Rahko, 1970). A modification on the earlier technique was the use of insert ear receivers for the recruitment test given above. The equipment was calibrated several times during the test period.

The test began with the audiological examination described above. The SISI test was

c II Average hearing thresholds with Bekesy audiometer (re 0 0002 dyn/cm<sup>2</sup>) and balance levels re 3 min 20 dB suprathreshold adaptation (SL) in control ear

Chronic otitis						Otosclerosis						Total material					
Hearing thresholds				Balance levels		Hearing thresholds				Balance levels		Hearing thresholds				Balance levels	
Adapt ear		Control ear		Control ear		Adapt ear		Control ear		Control ear		Adapt ear		Control ear		Control ear	
Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
518	150	144	108	32.8	12.5	503	132	172	117	34.9	12.6	513	143	153	110	33.5	12.5
333	12.6	43	8.2	23.9	12.3	317	107	17	8.4	32.7	14.9	330	12.0	3.7	8.2	26.7	13.7

administered on the conductively impaired ear 0 dB SL using 1 dB intensity pulses. After a short pause the patient balanced the 20 dB pulsed tone in the test ear with a similar tone in the control ear. The tone in the ear was then made continuous for 3 min, and the subject balanced it with a pulsed tone in the control ear and recorded the result. SISI increments were then added immediately to the adapted continuous tone in the test ear to be examined. The test frequencies were 500 Hz and 2000 Hz, and the masking was employed using 50-70 dB white noise (active level) in the control ear (Palva & Järä, 1962).

For the purposes of the statistical evaluation the threshold adaptation was measured 15, 45, 60, 90, 120, 150 and 180 sec after the start of the test. The reference value was the preadaptation balancing level of the control ear to the 20 dB SL tone given to the test ear. The results were processed at the University Computing Centre. After determination of mean values and standard deviations the differences between the averaged results were examined using the Student's test. Differences were considered significant when  $p < 0.01$ .

## RESULTS

The audiological findings from the basic tests given in Table I. The air conduction thresholds for the two groups do not vary much,

and though the 3 min threshold adaptation test showed slightly less adaptation in the control ear in both groups, the difference was not significant.

The recruitment phenomenon was incomplete in less than one-quarter of the cases with chronic otitis at 500 Hz and in one third at 2000 Hz. Incomplete recruitment occurred in two-fifths of the cases of otosclerosis at 500 Hz and in a quarter at 2000 Hz. Complete loudness balance was never observed at the 100 dB maximum level (SL) for the control ear, the partial recruitment ranging from 10 dB to 25 dB.

In the total series the SRT was 40.4 dB in the test ear and 9.4 dB in the control ear, while for the chronic otitis patients the figures were 40.4 dB and 11.8 dB, and for those with otosclerosis 41.3 dB and 10.8 dB, respectively. The average speech discrimination score for the total material was 96.8% for the test ear and 98.7% for the control ear, the intra-group differences being of the order of 1%. The average discrimination score in the filtered speech test was 82.9% for the test ear, 86.3% for the control ear and 81.9% binaurally. The sub-groups are too small to warrant comparison.

In the group analysed for suprathreshold adaptation the difference between the air conduction thresholds of the test ear and control ear averaged between 33.3 dB and 28.4 dB, depending on the frequency and group.

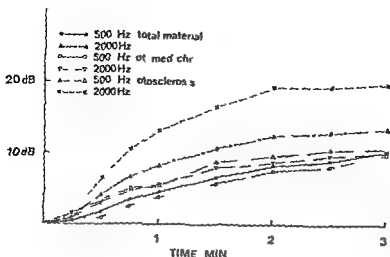


Fig 1 Perstimulatory suprathreshold adaptation as a function of frequency & time testing with sustained tone at SL.

Table II shows the mean thresholds measured with a Bekesy audiometer re 0 0002 dyn/cm<sup>2</sup> and the balancing levels in the control ear (SL) prior to adaptation, classified according to the type of defect and the frequency used. The lowest balancing level was recorded in the group with chronic otitis at 2 000 Hz. All the balancing levels exceeded 20 dB, the values being higher at 500 Hz than at 2 000 Hz.

Fig 1 shows the adaptation as a function of time. Maximum adaptation was recorded at 2 000 Hz in the otosclerosis patients. Adaptation seemed to occur within the first two minutes, after which time changes were small.

The mean SISI scores for the various test groups before and after 20 dB suprathreshold adaptation for 3 min are presented in Table III. The mean preadaptation values ranged from 15.0% to 31.1%, and the postadaptation values from 9.0% to 30.7%. On average there seemed to be a slight lowering in the SISI score, but due to the wide scatter found in the results, these

differences were not significant. Figs 2a show the individual results in the various groups before and after adaptation.

Analysis of the SISI values for each individual subject according to the Jerger classification (1961) shows that the negative and questionable groups were the largest before and after adaptation (Table IV). However, the proportion of positive values in the chronic otitis group was 26% at 2 000 Hz, and 22% in the total material. Positive values were more numerous generally at 2 000 Hz than at 500 Hz, though the proportion of positive results among the postadaptation SISI values seemed to be slightly smaller than elsewhere.

A transition from one Jerger group to another occurred in six of the 43 patients with chronic otitis at 500 Hz and in 2 000 Hz. The changes were evenly distributed in both directions. In the otosclerosis group the figures were 2 and 4 out of a total of 6, all the changes were in the diminishing direction.

Table III Average SISI scores before and after adaptation in different groups of the conductive hearing impaired

Frequency	Chronic otitis				Otosclerosis				Total material			
	Pre adapt		Post adapt		Pre adapt		Post adapt		Pre adapt		Post adapt	
	Mean %	S.D.	Mean %	S.D.	Mean %	S.D.	Mean %	S.D.	Mean %	S.D.	Mean %	S.D.
500 Hz	21.4	19.2	20.1	24.0	15.8	22.0	9.0	16.8	19.6	20.1	16.8	22.1
2 000 Hz	31.1	28.2	30.7	28.6	22.0	20.9	20.5	22.2	28.2	26.3	27.4	22.1

36% of the chronic otitis cases at 500 Hz, 76% at 2000 Hz no change was shown corresponding ratios for the otosclerosis were 90% and 80%, and for the total material 87% and 78%. No transition from positive to negative or vice versa was observed. It could thus be said that the majority of the values did not change their Jerger group after a 20 dB suprathreshold adaptation of 3

## DISCUSSION

Normal SISI scores in cases of conductive defects were defined in the original article of Jerger (1959) as lying in the range 0–15%. Jerger (1961), he reported 15 instances of a negative SISI score, 4 of a questionable score  $\pm 2$  of a positive score, in a material of 21 cases with conductive defects. The average adaptation values in the range 15–31% obtained in the present work are higher than those reported by Jerger, and a comparison of results for the total series here with those of Jerger (1961) shows that 22% of all cases in the present series gave positive values at 2000 Hz, as against approx 10% in Jerger's material. However, Jerger did not specify the frequency.

In the chronic otitis group the percentage of positive SISI values was higher, 36%, at 2000 Hz, which differs even more from Jerger's values. Questionable values at

Table IV. SISI scores grouped using Jerger's classification before and after 3 min 20 dB SL adaptation

Frequency (Hz)	Before adaptation			After adaptation		
	0–15%	20–55%	60–100%	0–15%	20–55%	60–100%
Chronic otitis						
500	21	11	3	22	17	3
2000	19	12	11	16	17	9
Otosclerosis						
500	14	5	1	13	5	0
2000	11	7	3	13	5	2
Total material						
500	35	23	4	37	22	3
2000	29	20	14	29	22	11

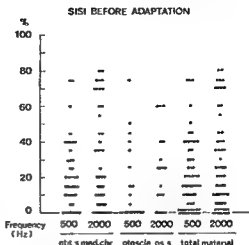


Fig 2 Individual SISI scores at various frequencies in the pre-adaptation test

2000 Hz occurred in 32% of cases, as against approx. 24% found by Jerger (1961). In both subgroups in the present series the 2000 Hz results differ from Jerger's values more than do the 500 Hz results.

Student's *t* test comparison of the present SISI values for conductive deafness with those found earlier (Rahko, 1971) in subjects with normal hearing revealed no differences between the preadaptation groups ( $p > 0.01$ ), but a significant difference at 500 Hz in the post-adaptation groups between the otosclerosis patients and the normal subjects ( $p < 0.01$ ).

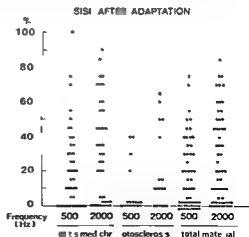


Fig 3 Individual SISI scores at various frequencies in the post-adaptation test



A very wide range of SISI values was found in the present study. Therefore, as pointed out earlier (Rahko, 1971), the results support the recommendations of Yantis & Decker (1964) that the limit for positive results should be raised to 80%, and of Hanley & Utting (1965) that an increment smaller than 1 dB should be used. On the other hand, partial recruitment, which has not as a rule been considered to be a feature of conductive deafness, occurred in 30% of the material at 2 000 Hz and in 29% at 500 Hz. There were no cases of mixed deafness, however, and the recruitment apparently is mechanical in nature (Anderson & Barr, 1966, Palva *et al.*, 1974).

Suprathreshold adaptation has not been studied earlier at the 20 dB SL in connection with conductive hearing defects, adaptation with 60 dB SL 2 000 Hz tone exceeded that at lower frequencies (Kärjä, 1970). The otosclerosis group in the present series is more in keeping with this trend, whereas the suprathreshold adaptation of the chronic otitis group was practically the same at both frequencies. A statistical comparison with the normal material (1971) revealed no significant differences in suprathreshold adaptation in any of the groups.

The effect of adaptation on the SISI test in cases of conductive deafness seems to be negligible. The mean values for the total material showed a slightly declining trend, but the change was not statistically significant. The difference was greatest in the otosclerosis group at 500 Hz, a decline from 15% to 9%, while other changes were considerably smaller. Individual transitions from one Jerger group to another were more frequent in both directions in the chronic otitis than in the otosclerosis group, where the trend was predominantly a declining one. There was not a single change from positive (60–100%) to negative (0–15%) or vice versa. 87% of the SISI scores at 500 Hz and 78% at 2 000 Hz did not change from one Jerger's group to another.

Subsequent to suprathreshold adaptation, the subjective tone level in the SISI test had fallen by more than 10 dB, and in otosclerosis

patients at 2 000 Hz by even more. Thus, this adaptation had no statistically significant influence on the SISI test. This agrees with Jerger's statement (1959) that a cortical adaptation in the SISI test does not affect its results. This statement can now be extended to apply to cases of conductive hearing defects, subject of any of the test groups reported. This concurs with the nature of the defect, in which no major adaptation is to be expected.

A similar study is being conducted on patients with hearing defects.

## ZUSAMMENFASSUNG

Die Wirkung der Adaptation des Ohres auf den Test wurde an 43 Patienten mit chronischer Ohrerkrankung und an 20 Patienten mit Otosklerose 500 und 2 000 Hz untersucht. Die durchschnittliche Adaptation betrug etwas über 10 dB. Nur die Otosklerosegruppe wies bei 2 000 Hz eine nach der kontinuierlichen Stimulation einen Wert von 20 dB auf. Beim Präadaptationsstest variierten durchschnittlichen SISI Werte zwischen 15% vor und postadaptatorisch zwischen 9% und 30%. Streuung der präadaptatorischen SISI Werte war und erschwerte die statistische Auswertung. Die Klassifikation von Jerger gab es bei 2 000 Hz 22% positive Werte im gesamten Material und 32% fragwürdige Werte auf derselben Frequenz. Die Adaptation verringerte die positiven Werte etwas, und zwar in zunehmender Richtung. Nur in der Otosklerose waren sämtliche Abweichungen in abnehmender Richtung.

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## SISI TEST AND ADAPTATION

## III Subjects with Perceptive Hearing Defects

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**Abstract** The effect of the adaptation of the ear on the SISI test was studied in 60 completely and 18 incompletely recruiting patients and in 5 patients with a verified acoustic neuroma at 500 and 2 000 Hz. The subjective suprathreshold adaptation to a 3 min tone of 20 dB (SL) ranged from 10 dB to over 20 dB depending on the frequency and type of defect. The pre adaptation mean SISI values in the recruiting groups ranged from 38 to 51 %, and the post adaptation means from 38 to 49 %. In retrocochlear lesions only one SISI value was questionable before and after adaptation, the others being 0 %. The dispersion of the SISI scores was considerable, a fact

positive, 4 a questionable, and 3 a negative score, and 37 non recruiting ears of which 16 had a positive, 2 a questionable, and 5 a negative SISI score.

Yantis & Decker (1964) found that at 500 Hz out of 18 ears with a retrocochlear lesion 16 had a SISI score of 0 %, and the remaining two 60 and 95 %, whereas of 42 ears with a cochlear lesion 22 had a SISI score of 0 % and most of the remaining 20 had a SISI score exceeding 80 %. At 2 000 Hz, in the material, the SISI score was 0 % in 13 retrocochlear lesions and 100 % in 32 cochlear lesions.

Owens (1965) described 95 cochlear lesions of which 91 had a SISI score exceeding 60 % one of the frequencies used, viz. 500 Hz, 2 000 Hz or 4 000 Hz. In 12 ears with retrocochlear lesions the SISI score was 0 %. In 7 patients the host tone faded away a few seconds, in 3 the tone faded away but patients heard 2-3 dB increments, whereas in two the tone did not fade away at all and patients heard the 2-3 dB increments but a smaller ones.

Hughes (1968) selected for his study 15 patients in whom the SISI host tone faded away but the SISI scores exceeded 75 % at two of the frequencies studied. Fifteen patients had either a vascular or Al-based cochlear lesion and three had an acoustic neuroma, later verified surgically.

Young & Harbert (1967) described 15 lateral, recruiting sensorineural flat type

be borne in mind

In his original article on the SISI test, Jerger et al (1959) reported also on the values he had obtained in perceptive deafness. In nine cases of Ménière's disease the scores were from 70 to 100 %, and 95 to 100 %, respectively, in 34 cases of presbycusis the scores varied from 0 to 100 %, and in three cases of retrocochlear lesions they were 0 % at 500 and 2 000 Hz.

In a later article, Jerger (1961) reported on 20 cases of unilateral Ménière's disease, all with a positive SISI score, on 11 cases of neuroma of the acoustic nerve including nine with a positive and two with a questionable SISI score, and on perceptive unilateral hearing defects of undefined diagnosis, as follows: 16 completely recruiting ears of which 13 had a positive, 2 a questionable, and one a negative SISI score, 22 partially recruiting ears of which 15 had a

cts in which the SISI scores exceeded 60%  
 a 20 dB sensation level, and 13 cases with  
 ological adaptation (over 20 dB) in which  
 rrdless of the performance level of the test,  
 SISI scores were with two exceptions 0-5%.  
 y stated that in conductive and sensorineural  
 ring defects, excepting those with pathological  
 ptation, the SISI test was positive if the  
 nd pressure level (SPL) entering the inner  
 exceeded 60 dB

uprathreshold adaptation in perceptive hear  
 defects has not been studied at a sensation  
 l of 20 dB, although Palva (1952) reported  
 suprathreshold adaptation was at its  
 test at 45 dB in recruiting hearing defects  
 in non recruiting ears at 20 dB

he duration of the SISI test is 1 min 40 sec  
 therefore, as is known from the supra-  
 shold adaptation studies by Palva (1952 and  
 4), Kärjä (1968) and Palva & Kärjä (1969),  
 siderable adaptation of the host tone must  
 place The present study investigated the  
 ad of the SISI scores in perceptive hearing  
 ects of different types, the effect of adaptation  
 these scores and compared them with the  
 a obtained earlier in normals and in con-  
 tion deafness (Rahko, 1971 and 1974)

## MATERIAL AND METHODS

material consisted of 83 subjects, 37  
 men and 46 men, mean age 43 years All had  
 unilateral perceptive hearing defect Five  
 ents had an acoustic neurinoma verified  
 ologically, or in one case, by contrast medium  
 ography None of these ears showed loud-  
 recruitment The completely recruiting  
 up of 60 patients contained 32 with Menière's  
 ease and 7 with a definitely vascular cochlear  
 on, the remainder having a lesion of unde-  
 ed etiology The incompletely recruiting group  
 18 patients contained two with Meniere's  
 ease and 4 with a vascular cochlear lesion,  
 remainder having a perceptive loss of un-  
 med etiology Recruitment was considered  
 nplete when with 100 dB (SL) the difference

in loudness levels between the ears was  $\pm 5$   
 dB A recruitment deficit of 10 dB in the test  
 ear, or more, at 100 dB (SL) was defined as  
 incomplete recruitment

Only those patients in whom the differences  
 between hearing thresholds did not exceed 50  
 dB were included in the analysis of suprathre-  
 shold adaptation The effect of overhearing was  
 minimized using the insert receivers in the in-  
 vestigation of suprathreshold adaptation (Palva  
 & Palva, 1962)

The basic audiological examination of the  
 patients was the same as described earlier  
 (Rahko, 1974) They were subjected to pure  
 tone and speech audiometry (Palva, 1952), to  
 a 3-minute threshold adaptation test, recruitment  
 test according to Fowler (ABLB) and hearing  
 thresholds and interaural differences permitting  
 to a filtered speech test (Palva, 1965)

The SISI test procedure was similar to that  
 described earlier (Rahko, 1974) The SISI test  
 was given to the ear with the perceptive hearing  
 defect at 20 dB sensation level (SL) using 1 dB  
 pulses After a short interval the patient balanced  
 the 20 dB (SL) pulsed tone given to his affected  
 ear with a similar pulsed tone in the control ear  
 The tone to the affected ear was then given  
 continuously for 3 min and the test subject  
 balanced it with a pulsating tone in the control  
 ear making a recording with self-recording audio-  
 meter Immediately after this the SISI increments  
 were added to the continuous adapted tone in  
 the tested ear The test frequencies were 500 and  
 2000 Hz During the SISI test masking in the  
 control ear consisted of 50-70 dB white noise  
 (Palva & Palva, 1962)

For statistical treatment, the suprathreshold  
 adaptation was measured at points 15, 30, 45,  
 60, 90, 120, 150 and 180 sec The reference level  
 used was the pre-adaptation level of balancing  
 the tone in the control ear with the 20 dB (SL)  
 tone given to the tested ear The results were  
 computerized at the Oulu University Computer  
 Centre After the mean values and standard  
 deviations had been determined the extent of the  
 differences between the mean results was studied  
 using the Student's *t* test The differences were

Table I *Average audiological findings of the perceptive deafness material*

Type and frequency (Hz)	Pure tone thresholds (dB ISO)				3 min tone decay test (dB)				Recruitment, % 100 dB SL in G Number of sub-	
	Ac		Ce		Ac		Ce		None	Incomple
	Mean	S D	Mean	S D	Mean	S D	Mean	S D		
Incomplete recruit- ment										
500	40.8	19.6	10.0	4.7	7.0	4.8	5.0	5.3	—	11
2 000	51.7	20.7	12.2	7.5	27.3	22.8	15.2	22.0	—	19
Complete recruit- ment										
500	47.6	17.9	11.9	7.4	8.2	9.7	3.4	4.9	—	—
2 000	44.0	16.3	10.8	9.6	21.1	25.5	16.6	25.1	—	—
Retrocochlear										
500	46.0	23.8	11.0	7.4	18.0	19.6	6.3	9.5	5	—
2 000	67.5	6.5	15.0	12.2	28.8	13.8	5.0	7.0	5	—

Ac = adapting test ear, Ce = control ear

classified as significant if *p* was smaller than or equal to 0.01

## RESULTS

The results of the basic audiological tests are seen in Table I. The pure tone thresholds of the groups differed little, apart from the retrocochlear group at 2 000 Hz. In the 3-min threshold adaptation test, the tested ear in all groups showed a slightly higher degree of adaptation than the control ear. The difference was greatest in the retrocochlear group. Recruitment was complete, depending on the frequency, in 59–60 cases and incomplete in 18–19.

The patients with incomplete recruitment at 2 000 Hz had a mean speech reception threshold

(SRT) of 46.1 dB in the tested ear and 44.2 dB in the control ear, and in the group with complete recruitment the mean SRT for the tested ear was 44.2 dB and for the control ear 41.2 dB. The corresponding mean speech discrimination scores were 64.7 and 96.4%, and 96.9%, respectively. In the retrocochlear group the SRT was 48.3 dB in the tested ear and 13.8 dB in the control ear, and discrimination scores 38.3 and 100.0%, respectively. The differences between the recruiting and non-recruiting ears were thus fairly small.

The filtered speech test could be carried out only in nine patients. The mean results, at 500 and 2 000 Hz, were grouped as follows: the tested ear and control ear and binaural results, in

Table II *Average hearing thresholds with Békésy audiometer (re 0.0002 dyn/cm<sup>2</sup>) and balance levels before 3 min 20 dB (SL) suprathreshold adaptation in control ear. Perceptive material*

Frequency (Hz)	Incomplete recruitment						Complete recruitment						Retrocochlear					
	Hearing thresholds (dB)			Balance levels (dB)			Hearing thresholds (dB)			Balance levels (dB)			Hearing thresholds (dB)			Balance levels (dB)		
	Test ear	Control ear	Control ear	Test ear	Control ear	Control ear	Test ear	Control ear	Control ear	Test ear	Control ear	Control ear	Test ear	Control ear	Control ear	Test ear	Control ear	Control ear
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D
500	50.2	22.9	17.4	12.1	33.3	12.0	56.4	16.4	19.6	11.7	35.9	17.8	41.0	11.5	8.4	8.7	4.4	4.4
2 000	47.2	17.7	9.4	8.4	35.4	16.2	39.4	16.5	7.5	10.3	33.0	15.3	61.3	11.7	7.0	4.4	4.4	4.4

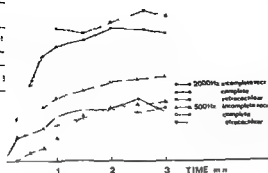


Fig. 1. Perstimulatory suprathreshold adaptation as a function of frequency and type of hearing defect during testing with sustained tones at 20 dB (SL).

complete recruitment group were 63.5, 80.0 and 68.0%, respectively, and in the complete recruitment group 76.6, 67.0 and 66.8%. The results were too few for statistical treatment. Table II shows the mean values for pure tone threshold measured with the Bekesy audiometer (0.002 dyn/cm<sup>2</sup>) and the balance levels in the ear before adaptation, grouped according to the hearing defect type and frequency. Figure 1 shows the adaptation as a function of time. Maximum adaptation was obtained at 2000 Hz in cases of retrocochlear hearing defect. Most of the full degree of adaptation seems to develop within the first 2 min. Adaptation at 500 Hz seems to exceed that at 2000 Hz in practically all groups. At 500 Hz it is of the order of 10 dB.

The mean SISI values before and after a 3 min suprathreshold adaptation at 20 dB are presented in Table III by test groups. The pre-adaptation values ranged from 38.0% to 50.9% in the re-

cruting groups and from 4.6% to 11.3% in the retrocochlear defect group. After adaptation the figures were 38.3% to 49.4% and 7.1% to 12.5%, respectively. The mean values of all groups increase, but no statistical significance is obtained owing to the great dispersion.

Figs 2 and 3 give the individual results graphically. As can be seen, the retrocochlear defect group contains only one value differing from 0%.

The analysis of the SISI values according to the Jerger classification (1961) presented in Table IV shows that the positive and negative values were most numerous in the recruiting groups, whereas the retrocochlear defect group contained only one questionable value, the others being negative. In the incomplete recruitment group, at 2000 Hz, the positive and negative SISI values before and after adaptation were equal in number, whereas at 500 Hz there were fewer negative than positive values. In the completely recruiting group the situation concerning the frequencies was the reverse. In the retrocochlear defect group there were no changes, and the majority of the SISI values were negative. Individually, transitions from one Jerger group to another are few, and 95–79% remained unchanged depending upon the type of defect and the frequency. No transition from positive to negative or vice versa was found.

## DISCUSSION

In his material of 17 patients with acoustic nerve trauma or Meniere's disease Jerger (1959) reported SISI values of 70–100% in cochlear

Table III. Average SISI scores before and after adaptation in different groups of perceptive material

Frequency	Incomplete recruitment				Complete recruitment				Retrocochlear			
	Pre-adapt		Post-adapt		Pre-adapt		Post-adapt		Pre-adapt		Post-adapt	
	Mean (%)	S.D.	Mean (%)	S.D.	Mean (%)	S.D.	Mean (%)	S.D.	Mean (%)	S.D.	Mean (%)	S.D.
2000 Hz	46.4	38.1	49.4	39.4	38.0	33.2	41.6	35.0	4.6	10.3	7.0	15.7
500 Hz	42.1	36.3	38.3	37.7	50.9	38.6	49.4	39.6	11.3	22.5	12.5	25.0

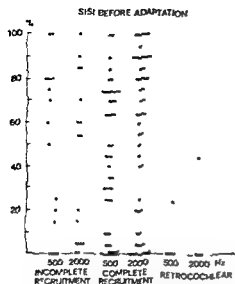


Fig 2 Individual SISI scores at various frequencies and in different types of hearing defects in the pre adaptation test

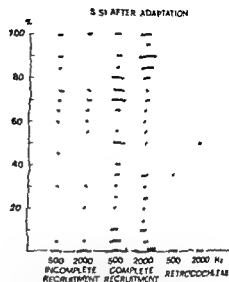


Fig 3 Individual SISI scores at various frequencies in different types of hearing defects in the post-adaptation test

defects. In a later article (1961) Jerger described 20 cases of Meniere's disease. Five of these patients had no recruitment phenomenon, and the SISI scores were positive in all cases. In the same study, Jerger listed 11 acoustic neuromas, the SISI test being negative in 10 and questionable in one. The SISI values obtained for the present retrocochlear defect group, viz. four negative and one questionable, agree with Jerger's results. On the other hand, the mean scores obtained in the recruiting groups in the present study, recruitment 46.4 and 42.1%, and complete recruitment 38.1% and 50.9%, are distinctly lower than Jerger's values. The SISI test was positive, according to the Jerger classification,

in the incomplete recruitment group of cases at 2000 Hz, questionable in 11% negative in 41%, and similarly positive questionable in 16%, and negative in 500 Hz. In the completely recruiting groups percentages were 53, 17 and 30 at 2000 Hz and 38, 26 and 36% at 500 Hz. Hence in the recruiting groups the SISI was positive most, in 53% and at least in 38% of the patients.

The results of the present study agree with those reported by Yantis & Decker (1961) as far as retrocochlear hearing defects are concerned, but differ concerning the cochlear defects for which they reported 83% positive values, 5% negative and the remainder questionable.

Table IV SISI scores grouped using Jerger's classification before and after 3 min 20 dB (S) threshold adaptation

Freq (Hz)	Before adaptation			After adaptation		
	0-15%	20-55%	60-100%	0-15%	20-55%	60-100%
Incomplete recruitment						
500	6	3	9	6	2	10
2000	7	3	7	7	3	7
Complete recruitment						
500	20	15	21	20	13	23
2000	17	10	31	20	9	29
Retrocochlear						
500	4	1	—	4	1	—
2000	3	1	—	3	1	—

at 500 Hz, and 92% positive and 2% negative at 2000 Hz. Similarly, the present results in the group of cochlear recruiting lesions those reported by Owens (1965a, b). In of these two studies the SISI test failed to positive in only 5 out of 95 cochlear lesions, as in one of the frequencies used, and in other study the SISI was positive in 70 out of 95 recruiting perceptive lesions, i.e. 97%.

In a comparison with those of earlier studies (Rahko, 1971 and 1974), using the Student's *t*-test, the pre-adaptation SISI values of the complete recruitment group at 2000 Hz are significantly bigger than those of otosclerosis and very significantly bigger than those of normal material. At 500 Hz, the mean pre-adaptation SISI values of the two groups of recruitment in the present material are very significantly bigger than those of the otosclerosis group and significantly bigger than those of the normal group. In post-adaptation SISI values, the complete and incomplete recruitment groups of this study also were highly significantly bigger at 500 Hz as compared with conductive defect groups, but at 2000 Hz in the complete recruitment group of this study SISI values were significantly bigger than those of the otosclerosis group.

The SISI values for the present group had a large dispersion. Positive values were obtained for 53–38% in the recruiting groups at different frequencies. On the other hand, in all of the retrocochlear lesions (acoustic neuromas) the SISI test was 0%. Hence, on the basis of the present and earlier results (Rahko, 1971 and 1974), it may be concluded that a single SISI value does not reliably make a diagnosis regarding the locus of pathology in hearing defects. Neither does a negative SISI distinguish different kinds of hearing defects solely from normal and from each other (Rahko, 1971 and 1974), but then the possibility of a retrocochlear lesion must always be carefully ruled out.

Suprathreshold adaptation in perceptive deafness has not previously been studied at the sensation level. In the present material,

adaptation in retrocochlear lesions was at its maximum at 2000 Hz, the host tone fading away in two cases. It was next greatest in completely recruiting hearing defects and smallest in incomplete recruitment. At 500 Hz the adaptation in all types of defects was of the 10 dB order. The decay of the host tone also agrees with Owens' results (1965) in seven of the 11 cases of an acoustic neuroma the host tone faded away. A comparison of the degree of adaptation with the normal and conductive defect materials (Rahko, 1971 and 1974) revealed no statistically significant differences between the groups.

Comparisons of the present mean SISI values in the various groups before and after adaptation reveal no consistent trend of change in either direction. The changes themselves are numerically small, and no statistically significant differences could be shown between the groups. The number of acoustic neuromas is naturally too small for a reliable comparison. A study of the results per individual reveals no transition from a positive to a negative Jerger group or vice versa. In the incomplete recruitment group at 500 and 2000 Hz 79% remained unchanged, as did 85% in the complete recruitment group at 500 Hz and 95% at 2000 Hz. The changes that occurred were fully symmetrical in both directions, except at 2000 Hz in the completely recruiting defect group where all changes were diminutions.

In the SISI test following a suprathreshold adaptation of 3 min at 20 dB (SL), the subjective intensity of the host tone had diminished by 10 to over 20 dB depending on the type of defect, and had completely faded away in two cases, but this did not seem to affect the SISI test significantly. This agrees with Jerger's (1959) claim that changes in loudness of the sustained host tone do not affect the result of the SISI test. This has in the present study been demonstrated to apply also to perceptive hearing defects.

## ZUSAMMENFASSUNG

Die Wirkung der Adaptation im Ohr auf den SISI Test wurde bei 60 vollkommen rekrutierenden und 18 un-



vollkommen rekrutierenden Personen sowie bei 5 zuverlässig verfierten Retrocochlearläsionen (Akusticus neurinom) bei 500 Hz und 2000 Hz untersucht. Die subjektive Grösse der Adaptation über der Schwelle bei 3 Min 20 dB (SL) variierte je nach der Frequenz und dem Fehlertypus von 10 dB bis über 20 dB. In den rekrutierenden Gruppen erstreckten sich die durchschnittlichen Werte bei dem Praadaptations SISI Test von 38% bis 51%, und bei dem Post-adaptations SISI Test von 38% bis 49%. Bei den Retrocochlearläsionen war der SISI Wert nur in einem Fall prä- und postadaptatorisch fragwürdig sonst 0%. Die Dispersion der SISI Test Werte war recht gross was die statistische Behandlung des Materials schwierig machte. Die Adaptation hatte keinen besonderen Einfluss auf den SISI Test Wert. Es konnte festgestellt werden dass hohe SISI Test Werte nicht zuverlässig Hörfehlertypen voneinander unterscheiden. Wenn der SISI Test Wert aber niedrig ist muss die Möglichkeit der Retrocochlearläsion immer beachtet werden.

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## THE USE OF THE DRILL IN EAR SURGERY

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The surgeon of today has at his disposal a large amount of technical aids. For the ear surgeon it is primarily the rotating drill which has helped him in the operating theatre. In spite of the fact that the drill is an important tool in daily operative procedures, problems concerning drills have been accorded surprisingly little attention in the medical press. It is noticeable when visiting various clinics in different parts of the world that there appears to be little correlation between surgical standards obtained and the quality of technical equipment used. The object of this paper is to illustrate some of those problems which exist concerning the use of the drill in oto-surgery.

*Cutting—a question of cutting*

Whether one works with an ordinary cutting burr, a finishing burr, or a diamond burr, the cutting principle is the same. Fig. 1 illustrates the sequence of events in front of the single

The cutting edge and its positioning against material which is to be cut can be characterized by three different angles. The bevel angle is the angle which is made between the cutting edge's facet surfaces. The clearance angle is the angle between the lower facet and the material which is to be cut. Finally, the rake angle is the angle between the upper facet and the plane which is at right angles to the material to be cut. The optimal positioning of the rake angle, bevel angle, and clearance angle, are known when used in industry for working both metals of different degrees of hardness as well as upon wood. However, the ideal conditions for cutting bone have not been determined. On the other hand, such an investigation is hardly necessary. The precision which is required in industrial work is mainly a result

of the economics of massproduction. Considerations as such are thus irrelevant as far as ear surgery is considered, where the limits for acceptable working conditions are relatively wide and rather different. Heat production must be minimised in order to prevent traumatising the surrounding bone tissue. This means that the rake angle must be relatively large and the bevel angle relatively small. The most common cause of excess heat production is without doubt the use of dull burs. The edges become dull either by heavy use or by corrosion. The situation can be avoided either by using cheap carbon steel drills (which rust easily) and throwing them away after each operation, or by using stainless steel drills or hard metal drills (corrosion resistant) for repeated use. Even with correct cutting speed, correct pressure, and a fine quality cutting edge, it is impossible to avoid considerable heat production unless the process is cooled by saline solution.

Another factor of importance is mechanically induced trauma. A cutting burr provided with high edges allows the surface to be worked faster, which is obviously an advantage in certain



Fig. 1 The sequence of cutting work

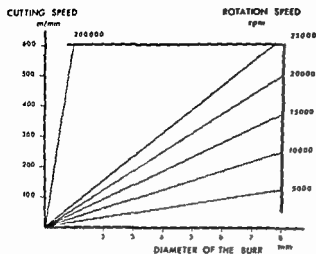


Fig 2 The relationship between the burr's diameter, the rotation speed and the cutting speed

stages of ear surgery. Such burrs, however, considerably increase the risk of mechanical traumatization of remaining bone as well as non-mineralized tissue. The otologist has long been aware of these risks. In more intricate phases of the operation he therefore uses a cutter with a shallow bevelled edge, such as a finishing burr. In this respect, diamond burrs of different stone size are a useful alternative.

#### Cutting speed—rotation speed range

Yet another important parameter in mechanical cutting work is the cutting speed, defined as the velocity with which the single edge is moved against the material to be worked. With a rotating burr it is the rotation speed and the diameter of the burr which determine the cutting speed. This relationship is illustrated in Fig 2.

As far as dentistry is concerned, two different areas of rotation speed have developed. High-speed rotors working between 200 000 and 400 000 rpm on the one hand, and on the other hand low-speed machines working with speeds up to 300 000 rpm.

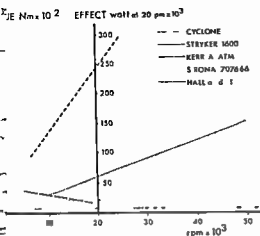
For the ear surgeon there is no question of choosing between these two different areas of rotation speed because of the relationship between the rotation speed and the large diameter which the rotation speed allows. If the rotation speed is exceeded, there is

of "throw off", i.e. that pieces of the edge break away and are thrown away. In ear surgery one must be careful with a burr diameter up to 8 mm for in the first stages of mastoidectomy. In terms this means that if one intends to use the same drill during the different stages of operation, one must choose the low speed.

There are still no published data which define the optimal cutting speed working with bone. Fig 2 illustrates how restricted to the low speed area (at 20 000 rpm) a burr with a diameter of 8 mm has a cutting speed of around 500 m per minute while with a diameter of only 1 mm has a cutting speed of 50 m/min. In order to obtain the cutting speed with the smaller burr diameter it would be necessary to increase the rotation speed from 20 000 to something over 100 000 rpm. Since the rotation speed of the rotor has a maximum of 20 000 rpm the operator must accept a cutting speed which is low. However, since this is not a question of increased production, the reduced working speed results from a lower cutting speed can be to combine rather well with the high pressure stages of an ear operation in which burrs of smaller diameter are used.

#### Effect

The most common way of relating the power of a drill is to describe its torque, which is usually described in Nm (Newtonmeter). The torque produced in the tangential power of the rotor produces multiplied by the distance from the movement's axle, i.e. the length of the drill. A different way of giving the power of a drill which is now used, is to define the so-called braking effect which is thus expressed in hp or horsepower. This gives a more readily understood figure describing the drill's ability to work against a resistance. The torque, as well as the braking effect, varies with the rotation speed of the drill. In the majority of cases the drill works at the maximum speed and it is the braking effect around 20 000 rpm which is most important.



The braking effect at different rotation speeds for the drills available

When working in ear surgery, the required braking effect varies considerably during different phases of an operation. When, for example, boring out a cell system with a thick temporal bone, one uses a large diameter burr under a powerful pressure in order to obtain the fastest working speed possible. In this stage the demands on the braking effect of the rotor are very high. On the other hand, when doing delicate work and using a small burr diameter, the requirements in this respect are very small.

Fig. 3 shows the braking effect and torque of a series of drills at present on the market are compared. An important difference between electric- and air-driven rotors is that the electric rotor with increased demands on torque becomes hot, which is a disadvantage if the rotor is in direct contact with the handpiece or thus in contact with the surgeon's hand.

### Handpiece

In general, two types of handpiece are used in ear surgery—a straight handpiece or an angled handpiece with a 20–30° angle. The function of the handpiece is to relay the movement from the motor to the head of the drill. Two factors are involved, firstly, the burr must be securely locked to the handpiece to avoid slipping when pushing forward. Secondly, the movement must be carefully centred in order to give high

working precision. It is widely accepted that the handpiece should be no wider than a pen in order to avoid obscuring the view of the working area. This means that one usually uses a handpiece designed for dental use. However, a characteristic of ear surgery is that in certain stages of an operation a large diameter burr is used under pressure in order to give the fastest possible working speed. This results in a considerable radial stress on the handpiece. The dimensions of a narrow handpiece result in a poor construction which is not designed for heavy, radially directed pressure. This means that the bearings of the drill receive an unusual amount of wear and tear, with consequent reduction of precision, i.e., increase in the radial throw-off and heating up of the handpiece. Such handpieces will obviously wear out quickly.

### Specifications required of a drill for use in ear surgery

There are a large number of drills to choose from on today's market. Apart from considerations of rotating speed and effectivity, several other factors should be appraised when making a choice. The size of the drill is determined principally by the construction of the drill itself. Today most of the older types of drill with belt transmission and rotating cable respectively have been replaced by smaller, electric- or air-driven rotors which are directly connected to the handpiece of the drill. In these machines, both size and weight are designed to be practicable to work with.

Another question of importance is the ease of sterilising the drill. Naturally it is an advantage if the drill as a complete unit can be autoclaved. The risk of contamination is reduced—especially important when changing handpieces. However, this obviously sets high demands on the specifications of both material and construction of the rotor, which reflects itself in the price of the equipment. Sometimes while operating, the burr catches in soft tissue whereby damage easily follows. From this point of view it would be an advantage if the drill can be stopped abruptly. Providing that the

NAME Type	SPEED rpm $\times 10^3$	BRAKING EFFECT at 20 $\times 10^3$ rpm watt	OPERATING MEDIUM	OPERATING PRESSURE N $\times 10^4$	FLOW $\frac{\text{liters}}{\text{min}}$	AUTOCLAVABLE
Cyclone standard	0 - 22	250	air	45	200	no
Cyclone-Kirurg W Weck air drill	0 - 25	250	air	50	170	yes
Hall air drill	0 - 100	8	nitrogen	70	180	yes
Stryker 1600	0 - 50	40	air	60	57	yes
Sirona 707666	4 - 40	23	electricity	-	-	no
Kerr A-ATM	0 - 20	6	electricity	-	-	no

Fig 4 Some properties of different drills. The Cyclone Kirurg W rotor is identical with Weck air drill

qualities described above are satisfactory, the deciding factors in the choice of a drill are reliability, durability and price. Quality in terms of reliability and durability by far outweigh a

higher price. Fig 4 illustrates data of drills obtainable at the present time.

#### Our solution

An objective comparison between different types of handpiece and drill has never been made. It would be difficult to carry out. Working conditions, economics, personal taste and special requirements vary considerably between different surgeons. It would therefore be wrong to try to give anything but general requirements when choosing a handpiece and drill for ear surgery.

However, we would finally like to state our choice, based on the conditions at present existing in our clinic. For the last 10 years we have worked with the Cyclone rotor (standard). The range of rotation speed is 0-20 000 rpm, the braking effect highly satisfactory (Fig. 4). The rotor cannot be sterilized and a sterile drape is thus used. On the other hand the unit is cheap and has shown itself to be highly reliable. By working together with the manufacturer we have developed a unit which consists of a footplate to which two rotors can be quickly attached (Fig. 5). One of the rotors has a standard straight handpiece, the other an angled handpiece. In this way it is always a handpiece at hand which is needed arising during any particular situation.



Fig 5 The unit consisting of a footplate and two rotors.



The straight handpiece equipped with strong bearing and chuck

tion The complete unit with two rotors is less than half the price of a sterilisable unit. Three units cover our needs adequately (approximately 500 operations/year). Thanks to the low cost of the unit, both the research labs and the residents' bone lab have been equipped with cyclone rotors. While working with the manufacturer we have also developed a special, straight handpiece. 6) This handpiece is autoclavable, has strong bearings which can stand heavy, applied pressure, and is also supplied with an especially strong chuck, which allows changes of burs and grips firmly enough at the end of the burr axle. This means of importance, in this way the head of the drill can be extended, the length depending on the situation during operation. In the first stage of a myringotomy, when a burr of wide diameter is used, and when a fast working speed is required and the radial stress is at a maximum, most of a burr as possible is used, i.e. the burr is maximally displaced into the handpiece. In the later stage of the operation—such as work in the antrum atticus region—burs of smaller diameter are used and the radial stress is small. At this time the burr can be displaced forward so that the chuck grips the end of the burr axle.

This means that instead of using a narrow handpiece, the drill head's axle is lengthened, which allows easy inspection and accessibility. The handpiece is cheap and proven to be of excellent durability. Only in certain stages of ear surgery—such as transmeatal work—has it proved necessary to use the angled handpiece.

## ZUSAMMENFASSUNG

Der Chirurg von heute verfügt über eine grosse Auswahl technischer Hilfsmittel. Dem Otolaryngologen hilft in erster Linie die Dentalbohrmaschine bei seinen operativen Einsätzen. Eine Bohrmaschine, bei der selten Betriebsstörungen auftreten und die nicht warmläuft, schenkt dem Chirurgen Zufriedenheit; eine schlecht funktionierende Maschine ist jedoch ein dauernder Grund zur Irritation. Obwohl die Bohrmaschine einen wichtigen Bestandteil bei täglichen operativen Einsätzen darstellt, ist dem Problem Bohrmaschine in der Fachliteratur bemerkenswert wenig Beachtung geschenkt worden. Es ist ebenso bemerkenswert, dass man, wenn man verschiedene Kliniken in der Welt besucht, keine höhere Korrelation zwischen dem chirurgischen Standard und dem Niveau der benutzten technischen Ausrüstung findet. Dieser Artikel möchte die existierenden Probleme behandeln, die Bohrmaschine in der Otolaryngologie beheben.

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## COCHLEAR DAMAGE RESULTING FROM KANAMYCIN AND FUROSEMIDE

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**Abstract** Permanent cochlear damage has been shown to occur in guinea pigs following the combined administration of kanamycin and furosemide. At the doses used, only a transient effect was measured with furosemide alone and no effect was detectable with kanamycin alone. This interaction results when a single subcutaneous dose of 400 mg/kg of kanamycin is followed in 2 hours by a single intravenous dose of furosemide. The dosage range for furosemide was 50 mg/kg for a just-detectable effect to 100 mg/kg for a very severe effect. Damage to the cochlea was ascertained by measures of the a.c. cochlear potential as well as surface preparation histology.

The aminoglycoside antibiotic kanamycin can interact with the diuretic ethacrynic acid to produce a severe permanent hearing loss. This interaction has been reported in patients (Mathog & Klein, 1969; Johnson & Hamilton, 1970) and in addition has been verified in experimental animals (West et al., 1973). The onset of deafness occurs shortly after the administration of the ethacrynic acid and if a sufficiently large dose of both drugs has been given the deafness is permanent. This permanent deafness is the result of hair cell destruction which has been demonstrated histologically in humans (Matz & Beal, 1969) and in experimental animals (West et al., 1973).

Furosemide is a potent diuretic drug related to ethacrynic acid. It has a different chemical structure, but its mechanism of action on the kidney is similar to that of ethacrynic acid. Also like ethacrynic acid, furosemide by itself, has been reported to produce temporary hearing losses in man (Schwartz et al., 1970) as well

as decreased auditory function in experimental animals (Mathog et al., 1970; Brown & Elvee, 1972). Unlike the case with ethacrynic acid there are no clinical cases reported which indicate that furosemide can interact with kanamycin to produce permanent deafness. Nonetheless, it was thought that such an interaction might be possible providing furosemide was given. The present investigation was designed to determine whether or not an interaction between kanamycin and furosemide would produce permanent cochlear damage in experimental animals.

### METHODS

This experiment has been divided into two parts. The first part demonstrates that an interaction between furosemide and kanamycin does exist and the second part demonstrates that the effect is permanent and related to the administration of furosemide.

In both parts of this experiment, the effect of the drugs on the ear was monitored by observing the cochlea's ability to generate an alternating current (a.c.) cochlear potential response to pure tone sound stimuli (Fettiplace & Lawrence, 1954). The electrical signal from the cochlea was amplified differentially 100 times and recorded using a General Electric Model 1900 wave analyzer. The sound stimuli were generated with a Western Electric Model 150 speaker. The sound intensity was measured in dB for each frequency for each animal with a

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attached to a  $\frac{1}{8}$ -inch B & K calibrated  
ophone (Vernon et al, in press). All sound  
intensities are referred to 1 dyne/cm<sup>2</sup>. Details  
of the methods employed for producing, and  
measuring the sound stimuli, as well as those  
used to record the a.c. cochlear potential and  
to rule out the possibility of radiation artifact,  
have been described before (Brummett et al,  
1972).

For the first part of the experiment, a total  
of 12 guinea pigs were used. They were anesthe-  
tized with dial (60 mg/kg) and urethane (240  
mg/kg) administered interperitoneally. A tracheal  
cannula was inserted, through which the animal  
was artificially respired. The rate and depth  
of respiration were so adjusted as to prevent  
spontaneous contractions of the middle ear  
muscles (Meikle et al, in preparation). Details  
of the methods employed to maintain body  
temperature, expose the round window mem-  
brane, insert and attach the round window  
electrode and keep the bulla free of accumulated  
fluids have been reported previously (Brummett  
et al, 1972; Mitchell et al, 1973). After the  
animal had been prepared, baseline data on  
cochlear function of each animal was obtained  
from the 1  $\mu$ V sensitivity function of the a.c.  
cochlear potential between 100 Hz and 20 kHz.  
In addition, the maximum amount of a.c.  
cochlear potential that the animal could generate  
between 1 kHz and 10 kHz was determined, and it will  
be referred to as an intensity function. Following  
baseline observations, a single 400 mg/kg  
dose of kanamycin was given subcutaneously.  
A period of 2 hours was allowed to elapse for  
kanamycin to accumulate in the perilymph  
of the cochlea. At the end of this 2-hour period,  
the 1  $\mu$ V isopotential sensitivity function  
and the maximum a.c. cochlear potential output  
between 1 kHz and 10 kHz were repeated to ascertain  
if cochlear function had not deteriorated.  
At this time, sufficient sound intensity at 1 kHz  
was delivered to the ear so that it would produce  
about 150  $\mu$ V of a.c. cochlear potential. This  
amount of sound is well below that needed to  
produce cochlear damage. Finally a single 100  
mg/kg dose of furosemide was administered

intravenously over a 1-minute injection period  
through an indwelling cannula in the ipsilateral  
jugular vein. In order to monitor any changes in  
cochlear function resulting from the administra-  
tion of these drugs, the a.c. cochlear potential  
resulting from the 1 kHz tone was continuously  
followed over a 3 to 4 hour time span.

As has been reported (West et al, 1973)  
for kanamycin and ethacrynic acid, the guinea  
pigs that had received both the kanamycin and  
furosemide developed not only a short duration  
loss of cochlear function, but in addition, a  
more profound effect occurred 2 hours after  
the furosemide. This second loss in the ability  
to generate the a.c. cochlear potential did not  
appear to return during the time course of this  
short term experiment.

A second study was designed to determine the  
permanency of this interaction effect. A group of  
4 guinea pigs were anesthetized with pentobar-  
bital (35 mg/kg) given interperitoneally. An  
indwelling jugular catheter was placed and the  
animal was prepared for recording a.c. cochlear  
potential in a manner so as to prevent post-  
operative infections (West et al, 1973). Both  
sensitivity and intensity functions of the a.c.  
cochlear potential were determined in one ear.  
After calibrating the sound system, the opening  
in the bulla was covered with crushed gelfoam  
and the skin closed with Michel wound clips.  
At this time, a single 400 mg/kg dose of kana-  
mycin was given subcutaneously. Two hours  
later, a single 100 mg/kg dose of furosemide  
was given intravenously over a 1 minute injection  
period. The indwelling catheter was removed  
and the skin closed with Michel wound clips.  
The animals were allowed to recover from the  
anesthesia and were maintained for 30 days in  
the animal quarters. In addition, 4 groups of 4  
animals each were treated in an identical fashion  
except that they received doses of 50, 60 or 70  
mg/kg of furosemide in order to establish a  
dose-response relationship for furosemide with  
a constant 400 mg/kg dose of kanamycin.  
After the 30 day recovery period, the animals  
were re-anesthetized with dial and urethane and  
sensitivity and intensity functions of the a.c.



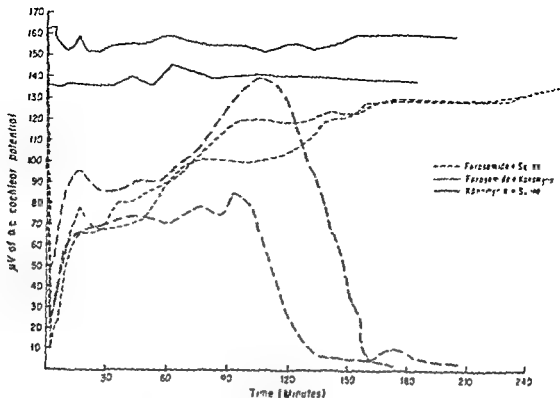


Fig. 1 Effects of furosemide (100 mg/kg) and kanamycin (400 mg/kg) on the a.c. cochlear potential that is being generated in response to a 1 000 Hz continuous tone. Kanamycin was given 2 hours prior to the furosemide.

cochlear potential were determined as described for the short-term experiment. However, in this case, both ears were evaluated. The temporal bones were saved for histology, the cochlea being perfused with 1% osmium tetroxide in Dalton's fixative. Surface preparation histology was performed to determine the presence or absence of both the inner and outer hair cells (West et al., 1973).

In both the short- and long-term experiments, control animals were run where the kanamycin dose was replaced with saline and the animal then had its normal dose of furosemide. Other control animals had their usual dosage of kanamycin, but the furosemide was replaced by saline. In either case, the volume of saline was equivalent to that of a proper dose of drug. In the case of kanamycin, the drug dosage is calculated as kanamycin base. The kanamycin sulfate solution contained 333 mg/ml of kanamycin base. For the furosemide, the drug concentration was 10 mg of furosemide per ml.

## RESULTS

Fig. 1 depicts the a.c. cochlear potential obtained at 1 kHz from the short-term experiment guinea pigs. In this instance the animal had received a single 400 mg/kg subcutaneous injection of kanamycin 2 hours prior to 100 mg/kg intravenous injection of furosemide. It can be seen that the ability of the a.c. cochlea to generate the a.c. cochlear potential to the continuous 1 kHz tone was rapidly depressed after this dose of furosemide. Depression was maximum in about 2-4 minutes whereupon it gradually returned toward control levels over a 2-hour time period. This drop in a.c. cochlear potential output and return to control values is typical of the effect of furosemide by itself. At this time and without any further drug administration the a.c. cochlear potential was again depressed. This depression was not followed by any recovery. Kanamycin by itself did not produce any effect on the a.c. cochlear potential when

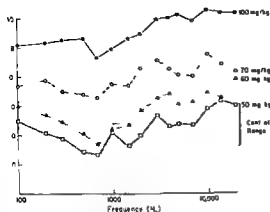


Fig 3 Mean  $1 \mu\text{V}$  sensitivity functions obtained 30 days after the guinea pigs had received a single  $400 \text{ mg/kg}$  intravenous dose of kanamycin followed in 2 hours by intravenous doses of furosemide. The control range from all animals (16) is shown in the shaded area. Mean data from the 8 ears of 4 guinea pigs for each dose of furosemide.

When followed by an intravenous dose of saline, the depression of the a.c. cochlear potential was permanent. In this case the animals were injected with kanamycin which was followed in 2 hours with the furosemide. In all cases control sensitivity and intensity functions were obtained just before the drugs were given. The animals were then surgically closed and allowed to recover for 30 days. After this time they were again

used to determine if this effect on the a.c. cochlear

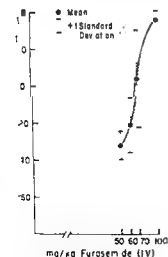


Fig 4 Dose-response relationship of a single dose of kanamycin followed by differing doses of furosemide on the maximum a.c. cochlear potential output at  $1000 \text{ Hz}$ . Each point represents the average of either 4 right ears or 4 left ears as indicated.

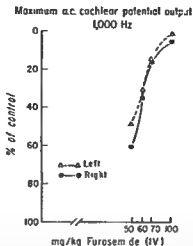


Fig 5 Dose-response relationship of a single dose of kanamycin followed by differing doses of furosemide on the maximum a.c. cochlear potential output at  $10000 \text{ Hz}$ . Each point represents the average of either 4 right ears or 4 left ears as indicated.

used to determine if this effect on the a.c. cochlear potential was permanent. In this case the animals were injected with kanamycin which was followed in 2 hours with the furosemide. In all cases control sensitivity and intensity functions were obtained just before the drugs were given. The animals were then surgically closed and allowed to recover for 30 days. After this time they were again

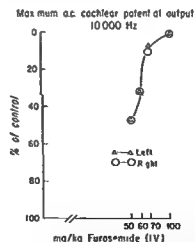
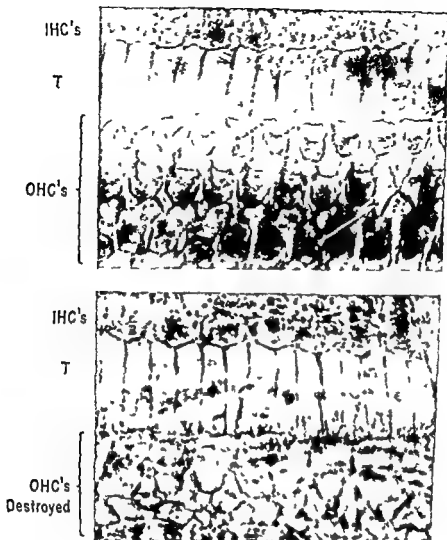


Fig 6 Dose-response relationship of a single dose of kanamycin followed by differing doses of furosemide on the maximum a.c. cochlear potential output at  $10000 \text{ Hz}$ . Each point represents the average of either 4 right ears or 4 left ears as indicated.



6 Upper A surface preparation from the 2nd turn of a guinea pig that had received a single 400 mg/kg of kanamycin followed by a 50 mg/kg dose of furosemide. This specimen shows a typical scar from a missing outer hair cell (OHC) that is indicated by the arrow. This appears to be a normal specimen. Lower A surface

preparation for the 2nd turn of a guinea pig that received a single 400 mg/kg dose of kanamycin followed by a 70 mg/kg dose of furosemide. This specimen shows a total loss of OHC's. (Outer hair cells—OHC's; inner hair cell tunnel—T)

evaluated electrophysiologically. Fig. 2 presents the  $1 \mu\text{V}$  isopotential sensitivity function for these animals. The control (pre-drug) data is shown in the stippled area. The mean data from the right ears of all animals that had received the different doses of furosemide are shown as single lines. As can be seen, the 50 mg/kg dose produced no effect. The 60 mg/kg dose produced a possible effect in the high frequencies whereas the 70 and 100 mg/kg dose produced considerable permanent effect when measured 30 days after drug administration.

When the sound intensity that is required to produce  $1 \mu\text{V}$  of a c cochlear potential at frequencies tested is averaged, one is obtained that we will call the "average sensitivity function." The averaged  $1 \mu\text{V}$  sensitivity function obtained from the group of guinea pigs that received the same 400 mg/kg dose of kanamycin but different doses of furosemide are shown in Fig. 3. These data indicate that as the dose of furosemide increases the effect on cochlear function increases.

In addition to the effect on the  $1 \mu\text{V}$  sens

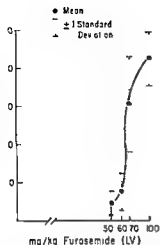


Fig. 7. Dose-response relationship of a single dose of furosemide on outer hair cells. Averaged data from the 8 ears of 10 pigs for each point.

ion of the a.c. cochlear potential, the maximum output of the cochlea was determined at 1 kHz and 10 kHz. For these determinations, sound intensity was increased in 5 dB while the a.c. cochlear potential that was generated by the cochlea was monitored and pressure produced a decrease in the cochlear potential. The maximum output from the intensity function determinations plotted in Figs. 4 and 5, as a percentage of control values that were obtained before administration. At 1 kHz, the mean control value was 1387  $\mu$ V and at 10 kHz it was 1000  $\mu$ V. The maximum values that were obtained at both the left and right ears are plotted separately. This shows that the effect on the a.c. cochlear potential is about equal bilaterally. It is also apparent from the data that the effect at 10 kHz was approximately equal to that at 1 kHz.

That is to say, the dose of furosemide that reduced the output at both frequencies to 50% of the maximum was about 50 mg/kg. Initially the number of outer hair cells were determined in an area from each turn of the cochlea. Both ears from each animal were counted and the percentage of missing hair cells were determined by counting the scars in

the cuticular plate resulting from hair cell destruction (Fig. 6).

It can be seen in Fig. 7 that the number of missing hair cells was also related to the dose of furosemide.

## DISCUSSION

Our data show that an ototoxic interaction can occur when both kanamycin and furosemide are administered to guinea pigs. This interaction occurs between 1½ and 2½ hours after a single 100 mg/kg intravenous dose of furosemide that is given two hours after a single 400 mg/kg subcutaneous dose of kanamycin. At this time the a.c. cochlear potential drops to extremely low values over a 45 to 60 minute time period. This drop appears to be independent and possibly produced by a different mechanism than that which produces the initial rapid fall in a.c. cochlear potential that follows an intravenous injection of furosemide. This initial rapid depression of a.c. cochlear function was monitored and there was no observed difference either in magnitude or time course between animals receiving furosemide alone or those receiving both kanamycin and furosemide.

This second drop in the production of the a.c. cochlear potential appears to be permanent. Animals that are examined 30 days after the administration of single doses of kanamycin followed by single doses of furosemide exhibit a permanently depressed ability to generate the a.c. cochlear potential. This change was seen in the sensitivity function of the a.c. cochlear potential as well as in the maximum amount of a.c. cochlear potential that could be produced at 1 kHz and 10 kHz. Furthermore, at this same time period, there are many hair cells missing from the organ of Corti which is also good evidence for a permanent cochlear effect.

When the single dose of kanamycin that was given to these animals is held constant and differing doses of furosemide were administered intravenously a dose-related ototoxic effect occurs. This dose-related effect was indicated by the a.c. cochlear potential as well as the number of missing hair cells.

In all parameters the measured dose effect curve was very steep in that small increments in dosage produced large effects on the cochlea. For instance, the 1  $\mu$ V sensitivity function of the a.c. cochlear potential indicated little or no change at 50 mg/kg of furosemide, but exhibited a very marked change at 100 mg/kg. Likewise the number of missing hair cells was only about 9% with a 50 mg/kg dose but 85% were missing with a 100 mg/kg dose. This steep dose response curve would mean that cochlear damage would go from little or no effect to severe damage with only a doubling of the dose.

One interesting observation is to be made when the dose effect curve for the averaged 1  $\mu$ V sensitivity function is compared to the dose effect curve for the maximum output. In the case of the sensitivity function, the 50 mg/kg dose of furosemide produced little or no change. At the same dosage, the effect on the maximum output of the a.c. cochlear potential was to reduce it to about one half of control values. It is possible that changes in the maximum output of a.c. cochlear potential is a more sensitive indicator of cochlear damage than is the 1  $\mu$ V sensitivity function.

This ototoxic interaction in guinea pigs with kanamycin and furosemide appears to be similar if not identical to that which has been reported for kanamycin and ethacrynic acid in guinea pigs. In the case of kanamycin and ethacrynic acid there are reports of similar interactions occurring in man when these two drugs are administered together. In the case of ethacrynic acid the dose that produced a very severe interaction effect was 40 mg/kg. In the case of furosemide a 50 mg/kg dose produced only a slight interaction effect. It may be that the lack of clinical reports is due to the fact that doses of furosemide that are large enough to produce the ototoxic interaction are not given clinically.

## ZUSAMMENFASSUNG

Es wurde gezeigt, dass die Kombination von Kanamycin und Furosemid dauernde Schädigung der Cochlea bei Meerschweinchen verursacht. Bei den angewandten Dosen bewirkte Furosemid allein nur eine temporäre

Schädigung, und nur mit Kanamycin wurde eine Wirkung beobachtet. Die kombinierte Wirkung zeigte sich nach einer subkutanen Injektion von 400 mg von Kanamycin und einer intravenösen Injektion von Furosemid zwei Stunden später. Eine Wirkung wurde gerade noch mit 50 mg/kg Furosemid beobachtet, während 100 mg/kg eine sehr starke Wirkung zeigten. Die Schädigung der Cochlea wurde durch Messung des Cochlea-Wechselstrom-Potentials und durch histologische Oberflächen-Präparationen

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## IMMEDIATE RESTORATION OF BASAL SENSORINEURAL HEARING (MB MENIERE) USING A PRESSURE CHAMBER

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**Abstract** A new technique for the restoration of basal sensorineural hearing loss in Mb Meniere was described. In 12 cases with unilateral basal sensorineural hearing loss, fullness of the ear, and tinnitus were reported. In the early stage of their disease the patients were treated in a pressure chamber where it was possible to increase or decrease the air pressure within the range  $\pm 110$  cm H<sub>2</sub>O. Equilibration of middle ear pressures to surrounding pressures was checked. Exposure to underpressure resulted in a rise of the hearing thresholds at low frequencies and relief of subjective symptoms. When the air pressure was increased the hearing thresholds were lowered and the sensation of tinnitus and fullness of the ear was accentuated. Changes in air pressure did not affect the healthy ear or the high frequencies in the middle ear. Hearing improvement attained at exposure to underpressure seemed to remain at atmospheric pressure level. The investigation was performed on the hypothesis that a distended membranous labyrinth might cause a venous congestion of the vestibular aqueduct leading to impaired endolymph absorption in the endolymphatic sac. Possible effects of changes in environmental pressure on the inner ear were discussed.

It is generally accepted today that endolymphatic hydrops is the basic feature of Mb Meniere. The theory is supported by histopathological findings in Mb Meniere (Hallpike & Cairns, 1938; Lindsay, 1942; Schuknecht, 1968; Gussen, 1970). A distention of the scala media with displacement of Reissner's membrane has been observed in many cases. In the vestibular part of the ear the distention mainly affects the saccule to a lesser degree, the utricle and the semicircular canals. In a patient with a basal sensorineural hearing loss without vertigo, Lind & Schulthess (1958) found a distention not only of the scala media

It was reported by Stahle (1968) that in 22% of cases of Meniere's disease the onset was without vestibular symptoms. Recurrent periods of basal sensorineural hearing loss, fullness of the ear and tinnitus were the clinical symptoms. It is not known how often the symptoms are restricted only to the cochlea and do not include vertigo.

In Meniere's disease, glycerol has been used for diagnostic purposes. It increases the osmotic pressure of the blood, which might induce a transient reduction of the endolymphatic hydrops. In 50% of the cases, improved hearing resulted (Klockhoff & Lindblom, 1967). Meniere's disease is a recurrent disease with fluctuating symptoms of fullness of the ear, vertigo, deafness and tinnitus. Thus, the endolymphatic hydrops is not permanent. Beside spontaneous changes it has been possible to affect the hydrops by changing the blood osmolality.

Investigations have been performed in order to evaluate the volume displacement of the middle ear mucosa as correlated to changes in the surrounding air pressure (Andreasson et al., 1975). When the surrounding air pressure was increased, the blood vessels of the middle ear mucosa were congested with blood to compensate for the relative underpressure in the middle ear cavity. With decreased air pressure it was possible to record a reduced filling of the blood vessels in the mucosa. Provided there was a total or partial obstruction somewhere in the mem-

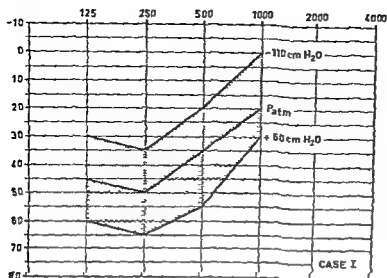


Fig 1 Case 1 Pure tone audiogram shows the hearing level before and after exposure to over and under pressure. Hearing threshold levels were less than 20 dB at exposure to overpressure (cm H<sub>2</sub>O). Short dashes Reduced pressure (-110 cm H<sub>2</sub>O) improved the hearing 15-20 dB. Dotted area

branous labyrinth it seemed possible to influence the volume-pressure relationship. Thus, a reduction of the surrounding air pressure might affect the blood volume of veins and capillaries in the inner as well as in the middle ear.

Three patients with unilateral acute symptoms of basal sensorineural hearing loss, fullness of the ear, and tinnitus were selected. The investigation was performed using a pressure chamber. The aim was to record the effect on hearing thresholds from increase and decrease of surrounding air pressure and try to aid the patients by a new therapeutic method.

#### Case histories

**Case no 1** B F Female, aged 39. No previous otological disease. On 1st October, 1974, symptoms started with tinnitus in her right ear. It remained for 3 weeks and was less pronounced

at the end of this period. On 20th October tinnitus returned together with fullness of the ear, slight vertigo, headache and impaired hearing.

**Case no 2** G B Female, aged 44. No previous otological disease. On 6th November, 1974, patient awoke with fullness of her right ear, tinnitus, and impaired hearing. No vertigo. Morning (Day 2) the symptoms were even more pronounced and the patient was admitted to ENT-clinic.

**Case no 3** R H Female, aged 48. Her mother probably had Meniere's disease. On 10th October 1974, the patient noticed the first symptoms: fullness of the left ear, impaired hearing, and an intense sensitivity to sounds. She was admitted to the ENT-clinic where a pure tone audiogram revealed a basal sensorineural hearing loss on the left ear. Within a few days the symptoms disappeared spontaneously, and

Table 1 Case 1

Time (min)	Pressure (cm H <sub>2</sub> O)	Fullness	Tinnitus	Vertigo	Pure tone audiogram Hearing loss (dB, ISO 1964) Dx			
					125	250	500	1000
0	P <sub>atm</sub>	+++	+++	0	45	50	35	20
8	+ 60	+++	+++	0	60	65	55	30
15	- 50	++	+++	0	40	40	30	10
21	- 90	+	++	0	30	30	25	5
26	- 110	++	++	0	30	35	20	0
33	P <sub>atm</sub>	+++	+++	0	45	50	35	15

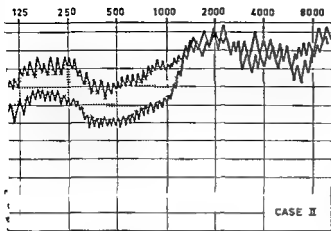


Fig 2 Case 2 Basal sensorineural hearing loss. Bekesy audiograms made immediately before and after the exposure to under-pressure. Dotted area shows the gain in hearing of 15 to 20 dB.

July the audiogram was quite normal. On December, 1974, the patient awoke with fullness, tinnitus, and impaired hearing in the left ear.

## METHODS AND EQUIPMENT

### Logical investigation

The treatment in the pressure chamber and the nature of the hearing loss was studied. Pure tone audiograms, acoustic stapedius reflex, acoustic decay, Fowler's test, hearing threshold level and speech discrimination test were performed. Electronystagmography was performed in the pressure chamber. An audiometer (Madsen, Model OB 70) and electroacoustic bridge (Madsen, Model ZO) were calibrated in accordance with ISO 1566 and ISO 1964 R389 (International Organization for Standardization) and checked to

fulfil the requirements of IEC Publication 177 (International Electrotechnical Commission, 1965).

Immediately before and after treatment in the pressure chamber, Bekesy audiograms, with interrupted tones, were performed. Conventional tracing where the frequency of the test signal moved gradually upward from 100 to 10 000 cps (Gransen Stadler, Model E-800).

### Pressure chamber

A pressure chamber with sound isolation was used. It was possible to increase or decrease the air pressure within the range of  $\pm 110$  cm H<sub>2</sub>O. Via a pipe system arranged in special valves the chamber was connected to a high pressure fan by which air could be blown into or evacuated from the chamber (Ingelstedt et al, 1967). The patient was placed in a sitting position inside the chamber. Pure tone audiograms were taken at

## II Case 2

Pressure (cm H <sub>2</sub> O)	Fullness	Tinnitus	Vertigo	Pure tone audiogram Hearing loss (dB, ISO 1964) Sin			
				125	250	500	1 000
$P_{atm}$	+	+	0	40	40	35	25
+30	+	+	0	30	30	35	15
-50	0	0	0	15	15	25	15
$P_{atm}$	+	+	0	20	25	25	15
$P_{atm}$	+	+	0	30	25	20	15
-50	0	0	0	20	25	15	5
$P_{atm}$	+	0	0	20	20	15	15



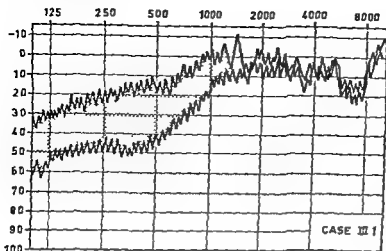


Fig 3 Case 3 Basal sensorineural loss. Bekésy audiogram performed before and after the stay in the pressure chamber. At the first exposure to the pressure there was a gain in hearing of 25 dB. Dotted area.

various pressure levels when the middle ear pressure was equilibrated to the pressure level of the chamber. The equilibration was checked with an electroacoustic bridge (Madson, Model ZO-72), where the minimum impedance was recorded when the ear drum was at its neutral position. All audiograms were performed when the fan was switched off. Pure tone audiograms at low frequencies were performed at 125, 250, 500 and 1 000 cps.

### RESULTS

In all 3 cases, pure tone audiograms showed a basal sensorineural hearing loss in the diseased ear and perfectly normal hearing in the other ear. The audiological investigation schedule revealed that the sensorineural hearing loss was located in the cochlea.

In all 3 cases, electronystagmography performed after treatment in the pressure chamber

showed a normal caloric response in both ears. During the increase or decrease of pressures, hearing thresholds of the non-diseased ear were not affected.

#### Case no. 1

The patient was subjected to both increased and reduced air pressure in the chamber.

Increased chamber pressure. When the pressure in the chamber was increased to +10 cm H<sub>2</sub>O (Table I), the patient complained of increased fullness in the diseased right ear. Hearing thresholds at low frequencies were depressed 20 dB (Fig. 1).

Reduced chamber pressure. When the chamber pressure was reduced, the patient complained of pain and fullness in the diseased ear drum. At a pressure level of -90 cm H<sub>2</sub>O, hearing thresholds at low frequencies

Table III Case 3

Time (min)	Pressure (cm H <sub>2</sub> O)	Fullness	Tinnitus	Vertigo	Pure tone audiogram Hearing loss (dB ISO 1964) Sin			
					125	250	500	1 000
0	$P_{atm}$	0	0	0	40	45	40	10
7	-50	0	0	0	40	45	40	10
49	-50	0	0	0	40	45	40	10
53	-70	0	0	0	40	40	30	5
62	-70	0	0	0	35	35	25	0
121	-70	0	0	0	30	30	20	0
129	$P_{atm}$	0	0	0	30	25	15	0
154	$P_{atm}$	0	0	0	30	25	15	0

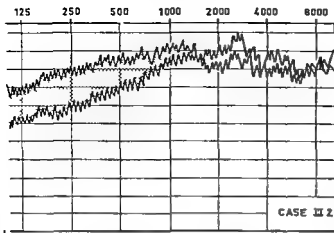


Fig 4 The same patient as in Fig 3. A recurrence occurred 11 days after the first exposure to underpressure. Bekeasy audiogram. The dotted area shows the gain in hearing.

10–20 dB compared with the audiogram at atmospheric pressure ( $P_{atm}$ ). A further reduction of chamber pressure to  $-110$  cm  $H_2O$  raised the hearing thresholds slightly (Fig 1). The patient had been exposed to reduced air pressure for only 13 minutes when it was increased to  $P_{atm}$ . At this level the audiogram was consistent with the original one at atmospheric pressure ( $P_{atm}$ ). The fullness of the ear had reduced.

#### Case no 2

On the second day (Day 2) after the onset of the disease the patient was treated for one hour in a pressure chamber at  $-50$  to  $-80$  cm  $H_2O$ . All subjective symptoms and the basal sensorineural hearing loss were not affected. Two days later (Day 4) the audiogram still had the same condition (Fig 2). The patient was exposed to reduced pressure and even at  $-30$  cm  $H_2O$  the sensation of fullness and tinnitus in the diseased ear vanished. After 24 minutes at  $-30$  and  $-50$

cm  $H_2O$  the gain in hearing thresholds at low frequencies was 10 to 25 dB (Table II). The pressure in the chamber was raised to atmospheric level ( $P_{atm}$ ) and the gain in hearing thresholds mostly remained. The patient was exposed to  $-50$  cm  $H_2O$  once more and the hearing thresholds were raised again (Table II). The Bekeasy audiograms performed before and after exposure to underpressure are shown in Fig 2. Two days later the audiogram was normal.

#### Case no 3

This patient was exposed to underpressure even on the first day (Day 1) of her disease. The treatment lasted for about 2 hours. After about 40 minutes the sensation of vertigo disappeared. The rise in hearing thresholds began somewhat later (see Table III). The hearing gain obtained in the chamber remained at  $P_{atm}$  and Bekeasy audiograms before and after the exposure to underpressure are compared in Fig 3.

On day 2 the Bekeasy audiogram was practi-

#### IV Case 3

Pressure (cm $H_2O$ )	Fullness	Tinnitus	Vertigo	Pure tone audiogram Hearing loss (dB ISO 1964) S =			
				125	250	500	1000
$P_{atm}$	++	+	+	30	30	15	0
-70	+	0	+	25	25	10	0
-70	0	0	+	25	25	10	0
-90	0	0	0	20	15	10	0
-90	0	0	0	20	20	10	0
$P_{atm}$	0	0	0	20	20	10	0

cally normal. On the following day (Day 3) all symptoms had returned but the hearing loss was less pronounced. After exposure to underpressure of  $-70$  and  $-90$  cm H<sub>2</sub>O for one hour (Table IV) there was a marked rise in hearing thresholds (Fig. 4). One day later (Day 4) the audiogram was normal again.

## DISCUSSION

Production and reabsorption of the endolymph seem to be the most essential points in the development of endolymphatic hydrops. If the endolymphatic sac is of vital importance in the reabsorption of endolymph (Guild, 1927) the endolymphatic duct, from the anatomical point of view, is the place most easily liable to obstruction. The vestibular aqueduct is a bony canal which contains the endolymphatic duct. In the proximal part, near the vestibulum, it narrows into an isthmus (Ogura & Clemis, 1971). The endolymphatic duct is surrounded by loose connective tissue containing blood vessels. The endolymphatic duct and sac probably derive part of their arterial blood supply and venous drainage via the paravestibular aqueduct (Anson, 1965; Ogura & Clemis, 1971).

From experimental studies it is known that endolymph is reabsorbed in the endolymphatic sac (Lundquist, 1965). Kimura & Schuknecht (1965) and Kimura (1967) blocked the endolymphatic duct in guinea pigs. Subsequent endolymphatic hydrops was observed. In microscopic investigations, abnormalities in the vestibular aqueduct have been observed by Altman & Zechner (1968) who observed a bony occlusion of the duct. By means of radiography Clemis & Valvassori (1968) showed a partial or total obliteration of the vestibular aqueduct. Further histological studies on temporal bones performed by Ogura & Clemis (1971) and Yuen & Schuknecht (1972) gave contradictory results. They did not find any differences in the calibre of the vestibular aqueducts in ears with Meniere's disease as compared with normal ears. Most histopathological investigations of Meniere's disease showed a distention of the membranous

labyrinth. Even in the original description of fibrosis of the walls of the endolymphatic sac was described (Hallpike & Cairns 1938) performing decompression operations in the endolymphatic sac, Shambaugh et al. (1968) observed ischemia of the exposed wall of the sac. In many cases partial or complete obliteration of the lumen of the sac. Microscopic examination showed a reduced vascularity and a marked epithelial fibrosis.

Thus, there seems to be evidence indicating that Meniere's disease is caused by a temporarily total obstruction of the endolymphatic duct. Increased intralabyrinthine pressure may result in reduced venous drainage. Venous stasis may affect the flow in the lymphatic duct by partial or total obstruction of the duct. In previous studies it has been shown that when the environmental air pressure is raised, blood vessels in the middle ear are filled with blood because of the increased pressure. Underpressure in the middle ear cavity causes the vessels to be emptied when the body is exposed to underpressure (Andreasson et al. 1971). There seemed to be many parallels between the middle ear cavity and the inner ear. It might also be possible to affect venous capillary blood filling in the inner ear.

Three patients with suspected acute endolymphatic hydrops are presented and the changes in hearing thresholds after exposure to increased and decreased air pressure are described. They all had variable, basal sensorineural hearing loss with tinnitus and fullness of the affected ear. In all cases it was possible to affect hearing thresholds by changes in the environmental air pressure. Exposure to reduced air pressure induced an increase in hearing thresholds. The improvement was, on average, 15–20 dB at an air pressure of 50–100 mm H<sub>2</sub>O, and in 2 cases the gain in hearing thresholds with the return to atmospheric pressure was as illustrated by Bekesy audiograms performed before and after the spell in the pressure chamber. In only one case (Case 1) was a further pressure applied. It resulted in an immediate decline of hearing thresholds at low frequencies and the subjective symptoms of fullness

us were accentuated. Symptoms and hearing thresholds again improved with the return of atmospheric pressure. Exposure to underpressure resulted in nearly complete relief of all active symptoms and improved hearing. In some cases the gain in hearing thresholds did not return, but hearing returned to the initial level of atmospheric pressure.

Diagrams performed in the pressure chamber were done when the pressure in the middle ear was equilibrated to the level of the pressure in the outer ear. The possibility exists that pressure changes in the inner ear are caused by a volume displacement via the round and oval windows. In a study on human temporal bones it was possible to quantify the volume displacement in the inner ear caused by changes in middle ear pressure. The recorded volume displacements were small at the pressure of 30–50 cm H<sub>2</sub>O (Lund & Pedersen, 1975). It is therefore conceivable that changes of the surrounding air pressure will affect the blood volume of the inner ear vessels.

It is not yet possible to quantify the volume change in the inner ear fluids caused by changes in blood congestion. Experimental studies on animals, however, are in progress in order to solve this question.

The endolymphatic duct and sac as well as sensory epithelial cells. The question arises whether it is possible to affect all cases with suspected endolymphatic hydrops by changing the surrounding air pressure.

In our experience, cases of Meniere's disease with sensorineural hearing loss without vertigo are improved by exposure to underpressure only during the acute stage of the disease and recurrence. Sensory cells and membranes damaged during standing hydrops will not respond to this treatment.

There are many questions about Meniere's disease which cannot be answered today. We think that the method presented is an improvement which will make it possible to help the

patient and open up new ways in the investigation of Meniere's disease.

## ZUSAMMENFASSUNG

Darstellung einer neuen Methode zur Behandlung von endolymphatischem Hydrops. Es werden drei Fälle von einseitiger Innenohrschwerhörigkeit mit Hörverlust für tiefe Töne, Druckgefühl und Ohrgeräuschen beschrieben. Die Behandlung erfolgte im akuten Krankheitsstadium in

und subjektive Symptomlinderung. Drucksteigerungen hingegen resultierten in einer Herabsetzung der Schwellenwerte und Intensivierung der Druckgefühle und Ohrgeräusche. Die Änderungen des Luftdrucks waren ohne

suchung wurde mit der Hypothese durchgeführt, dass eine Schwellung des membranösen Labyrinths venöse Stockungen im aqueductus vestibularis verursachen könnte, die einen verschlechterten Abfluss der Endolymph im

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## RESPONSES OF PERIPHERAL VESTIBULAR NEURONS TO ANGULAR AND LINEAR ACCELERATIONS IN THE SQUIRREL MONKEY

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Peripheral neurons innervating semicircular canals can respond to constant linear accelerations. It is presented that, in our preparation, the response is actual and arises from thermal gradients introduced by surgical exposure. Otolithic neurons do not respond to intense angular accelerations. Canal plugging alters the response of the corresponding afferents to linear acceleration, without obviously affecting resting activity. The procedure does not prevent the canals from responding to linear accelerations. The latter response, that seen in intact canals, is not due to thermal effects and may be related to the mechanisms underlying the persistent component of barbeque nystagmus.

Semicircular canals are commonly thought of as sensors of angular accelerations, the otolith organs as sensors of linear forces. This concept has gone unchallenged. Ledoux (1949) observed that canal neurons in the frog were sensitive to head tilt and his observations have recently been confirmed by Lowenstein (1972). In 1950, Gernsback presented evidence that the response of canal-related neurons in mammals could be modified by centrifugal force. Brown et al. (1970) found that rotating linear accelerations could affect the discharge of canal-related neurons in the cat vestibular nuclei and it is suggested that this response provided a physiological basis for the persistent component of barbeque nystagmus. In addition, several investigators (Benson & Barnes, 1973; Lowenstein, 1974) have proposed that the oto-

lith organs might be sensitive to angular accelerations, since such accelerations could cause a torsional motion of the otolithic membrane relative to the macula.

A primary purpose of the present study was to investigate the possibilities that the mammalian semicircular canals could respond to linear accelerations, the otolith organs to angular accelerations. During the course of the study, we also had occasion to investigate the response of peripheral neurons innervating canals which had been inactivated by the plugging procedure of Money & Scott (1962).

### METHODS

Fourty four adult male squirrel monkeys (*Saimiri sciureus*), anesthetized with sodium pentobarbital (15 mg/kg), were used. Body temperature was maintained at 36-38°C. Surgical and recording procedures were identical to those described previously (Goldberg & Fernandez, 1971). The left vestibular nerve was exposed by removal of the posterior part of the cerebrum and a small portion of the cerebellum. The nerve was covered with a 3% solution of agar-agar dissolved in physiological saline and a chamber mounted over the exposure. The chamber was filled with mineral oil. The microelectrode—a glass micropipette filled with 3M NaCl—was advanced by means of a screw micrometer mounted

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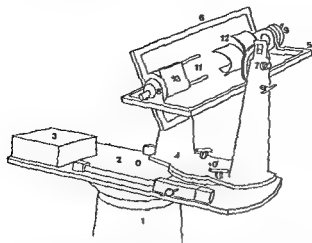


Fig. 1. Diagram of stimulating device. 1 rotating device; 2 horizontal platform; 3  $\times 1000$  amplifier; 4 superstructure mounted on guide rails. As shown here, superstructure yaw axis (0) 1 ft from horizontal platform axis (5). The superstructure's outer frame (5) pivots around pitch axis (7), the inner platform (6) pivots around roll axis (8, 9). 10 negative-capacitance preamplifier; 11 rails for attaching animal head holder so that center of head coincides with intersection of superstructure's pitch, roll and yaw axes. Center of head defined as intersection of interaural axis and midsagittal plane. Signals pass from animal to horizontal platform via slip-ring assemblies mounted on the three superstructure axes and then pass from horizontal platform by way of a slip ring assembly in the rotating device.

on the top of the chamber. Temperatures within the chamber were measured with thermistors (Yellow Springs Type 511) implanted through small holes drilled in the skull and then sealed with dental wax. In two experiments, thermal gradients in the chamber were controlled. Two 9-ohm, 2-watt wirewound resistors served as heaters. They were mounted on the inside walls of the chamber, one near the top of the mineral oil column, the other near the bottom. The resistors were powered by a d.c. battery and the current through them was controlled by a potentiometer. The superior canal was plugged in 6 of the animals. A small hole was drilled in the eminentia arcuata and the exposed membranous canal compressed with a mixture of bone dust and bone wax.

The animal was placed in a superstructure mounted on guide rails, which in turn were fixed to the horizontal platform of a rotating device (see Fig. 1). The superstructure could be moved

along the rails so that the animal's head was centered either over the axis of rotation or at an eccentric position up to 1 ft off axis. The superstructure could also be pivoted in the superstructure around pitch, roll or yaw axes. By convention, the zero tilt position was such that the lateral canal was in the horizontal plane with the animal's nose pointing towards the axis of rotation. The motion of the rotating device was controlled by a velocity servomechanism (Model 823). Centrifugal force and signals were used to study the response to linear acceleration. In the centrifugal force experiments, force trapezoids were used with the animal 1 ft off axis. The five periods of the trapezoid (T1-T5) are characterized as follows. A background velocity  $V_0 = 90^\circ/\text{sec}$  ( $\approx 0.077 g$ ) was maintained during T1 and T5. During T3, the velocity was at a higher value  $V_f = 360^\circ/\text{sec}$  ( $\approx 1.23 g$ ). During the transition periods T2 (and T4) the velocity increased (decreased) in such a way that the centrifugal force changed in a linear manner.

As a preliminary step, we examined the response of each unit to angular acceleration to various head tilts. Neurons innervating particular semicircular canal were easily identified because they responded to angular acceleration when the plane of the canal was tilted into the plane of motion. Units were considered otolith neurons if they proved unresponsive to angular accelerations, but were sensitive to tilt. Note that the classification scheme did not exclude the possibility that head tilts could also affect the discharge of canal neurons. Indeed, as we shall see, this was a common occurrence.

## RESULTS

### *Response of semicircular canal neurons to linear forces*

Fig. 2 illustrates the response of a posterior canal neuron to both angular and linear acceleration. Force trapezoids are used and the plane of motion of the ipsilateral (left) posterior canal is in the plane of motion. There are two components to the response. The angular component is most prominent during the periods of velocity transition

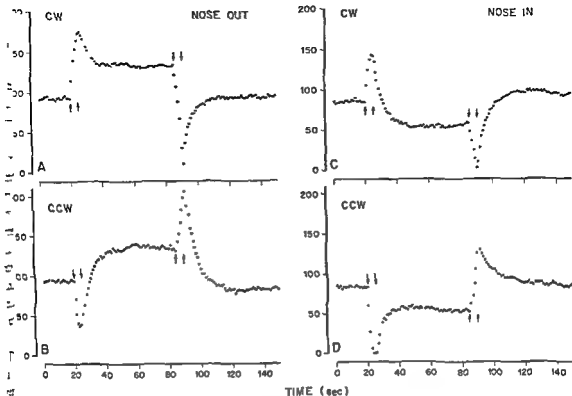


Fig. 2. Response of left posterior-canal neuron to force (rotorids, posterior canal in plane of motion ( $90^\circ$  lateral roll,  $45^\circ$  backward pitch)) A and B  $180^\circ$  yaw, C and D  $0^\circ$  yaw. In this and other figures, unless

otherwise stated, T1 = 20 sec, T2 = 5 sec, T3 = 60 sec, T4 = 5 sec, T5 = 60 sec, arrows (or bars) mark periods of velocity (force) transition, each point, discharge rate for 1 sec period.

reverses direction when the rotation is changed from CW to CCW. As expected, no reversal of angular component is seen when the animal is yawed  $180^\circ$  with respect to the rotation (cf Fig 2, left and right). The second component is a response to centrifugal force, as indicated by three facts. First, the response persists throughout the period of constant velocity. Second, there is no response reversal when rotation is changed from CW to CCW. And third, the response is reversed from excitatory to inhibitory when the animal is yawed  $180^\circ$ . To minimize the influence of angular acceleration, the animal was positioned so that the posterior canal was orthogonal to the plane of motion (Fig 3). Again responses to centrifugal force are evident in both yaw positions. Embedded in the responses are small angular components, presumably the result of a slight misalignment of the animal. The uncontaminated

response to centrifugal force can be obtained by averaging the CW and CCW responses for each yaw position (Fig 3, bottom). The excitatory (bottom left) and inhibitory (bottom right) reconstructions have similar time courses and magnitudes. The build-up and decline of the responses are both consistent with a first-order lag system having an approximate time constant of 5 sec. These relatively slow dynamics may be taken as evidence that the response to linear accelerations involves a movement of the cupula and endolymph.

Static tilts were used to characterize the directional selectivity and magnitude of the response to linear forces. Studies were carried out in 47 superior-canal neurons, 48 posterior-canal neurons, and 47 horizontal-canal neurons. Superior-canal neurons were activated by contralateral rolls and backward pitches, posterior-canal neurons by contralateral rolls and forward pit-



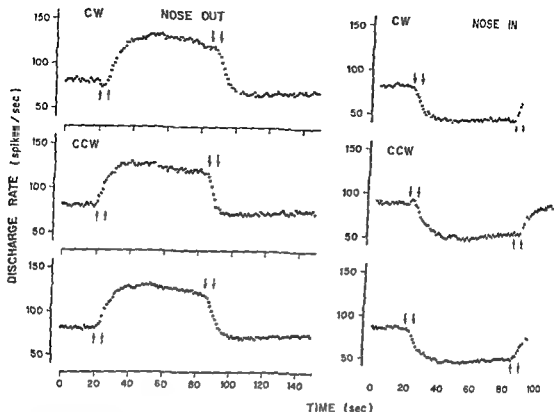


Fig. 3. Response of same posterior-canal neuron as in Fig. 2 to force trapezoids, animal in horizontal position. Left 180° yaw. Right 0° yaw.

ches, and horizontal-canal neurons by ipsilateral rolls and forward pitches. These rules held for over 90% of the vertical-canal neurons studied and for approximately 80% of the horizontal-canal neurons examined. The response magnitudes can be expressed in terms of a sensitivity factor—the maximum response expected to a 1-g acceleration (Fernández et al., 1972). The mean sensitivities ( $\pm$  S.E.M.) for neurons innervating the three canals were: superior canal,  $26.0 \pm 2.1$  spikes/sec; posterior canal,  $16.9 \pm 1.6$  spikes/sec; and horizontal canal,  $11.0 \pm 1.0$  spikes/sec.

The results could be explained were there a thermal gradient across the labyrinth. To investigate this possibility, thermistors were implanted on the top of the superior canal and on either the vestibular or trigeminal nerves. Measurements were made in 5 animals. A thermal gradient of 0.5–2.0°C was observed, in all cases, the top of the canal was colder than the deeper location. Undoubtedly, the gradient is

due to a partial equilibration of the m in the chamber with room air. The dir the gradient is consistent with the di selectivity of the responses.

An attempt was made in 2 animals to the gradient by means of electric heaters planted in the chamber. Fig. 4 presents from a superior-canal unit. At the start of the experiment, the heaters had been adjusted to eliminate the gradient and no response was observed (Fig. 4A). The heaters were then turned off and a gradient of about 1.5°C developed over the next several minutes. The corresponding response (Fig. 4B) was typical of that of more sensitive superior-canal units. Heat was then resumed, both the gradient and the response reversed direction (Fig. 4C). As a step, the gradient was again eliminated and the response considerably reduced (Fig. 4D). These results are consistent with Young's (1973) systematic observations and suggest the

ured thermal gradients are adequate to in both the direction and magnitude of the -acceleration response.

#### Use of otolith neurons to angular

#### Accelerations

Fig 5 compares the response of an otolith neuron to angular and linear accelerations. The stimulus was a velocity sinusoid with an acceleration magnitude of  $360^\circ/\text{sec}^2$ . With the animal tilted over the axis of rotation, no response was evident in either the response (Fig 5A) or histograms (Fig 5C). The lack of response is not attributable to an unfortunate positioning of the animal relative to the plane of rotation. Results, comparable to those obtained in the zero-tilt position, were also seen when the animal was pitched or rolled by  $90^\circ$ . In contrast, when the animal is moved to an upright position, a clear response is observed (Fig 5B and D). The response has a frequency that of the stimulus, as would be expected for the centrifugal-force profile resulting from a velocity sinusoid. In Fig 5E, force trapezoids are used. Again there is a response only in the upright position. The response illustrates that otolith neurons have relatively rapid dynamics. The contrast between the response dynamics of otolith and canal neurons can be seen by comparison of Fig 5E with the reconstructed responses at the bottom of Fig 3.

Essentially identical observations were made when otolith neurons were studied. Based on a previous classification scheme (Fernández et al., 1972), six units were assigned to the superior vestibular nerve and presumably innervated the utricle. The seventh was assigned to the inferior nerve and most likely was a saccular unit. In none of the units was a periodic response seen to sinusoidal angular accelerations. The only hint of a response was a small increase in spikes/sec in the average discharge occurring during the sinusoidal stimulus.

#### Use of neurons innervating plugged canals

In all animals, the superior canal was inactivated by plugging. A total of 160 vestibular units were

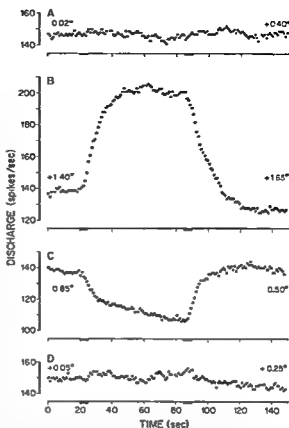


Fig 4 Response of superior-canal neuron to CW force trapezoids, animal in zero-tilt position. Temperature differences measured between trigeminal nerve and top of superior canal: positive values, trigeminal nerve warmer, measurements made before and after each rotation.

isolated. None of the units responded to angular accelerations in the plane of the superior canal. Sixty of the units could be assigned to the horizontal or posterior canals, 53 were classified as otolith neurons, since they were not influenced by angular accelerations, but did respond in a brisk manner to rapid head tilts. The remaining 47 units were considered as innervating the superior canal and, of these, 16 were studied in some detail. The resting discharge of the 16 units ranged from 5 to 120 spikes/sec with a mean ( $\pm$  S.E.M.) of  $82.5 \pm 8.8$  spikes/sec. These figures are similar to those characterizing units innervating unplugged canals (Goldberg & Fernandez, 1971).

Though the units were unaffected by angular accelerations, they did respond to linear acceleration.

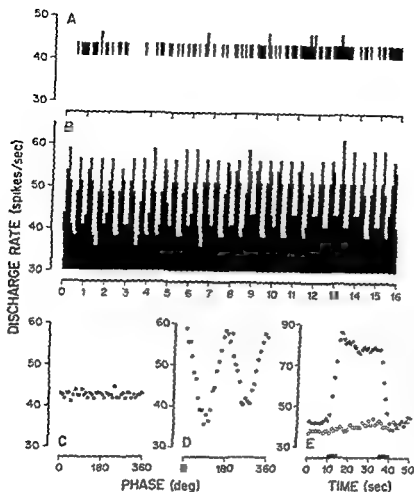
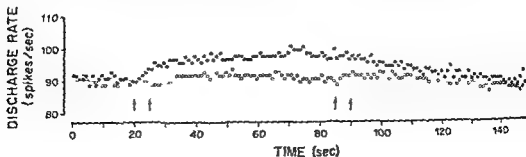


Fig. 5. Response of an animal in zero-hlt. A: 0.257 Hz velocity response histogram, B: phase histogram, head 1 ft scissa in units of (3 ft scissa) size of bin, C and D: phase histograms corresponding to A and B. E: maximum CCW bins/cycle. E: CW (T1-T5=10 sec, T2-T3=20 sec).  $\circ$  head-rotation axis,  $\bullet$  head.

tions. Force trapezoids were used. The animal was positioned so that the plane of the superior canal was in the plane of motion. Response was studied in two positions. In the first (or S1) position, the animal was rolled  $90^\circ$  towards the contralateral side and then pitched forward  $45^\circ$ . The second (or S2) position was identical except that the animal was also yawed  $180^\circ$ . In the S1

position, the animal's nose was pointed towards the rotation axis, in the S2 position the nose was pointing outwards. A typical unit response is illustrated in Fig. 6. Excitatory responses obtained in both S1 and S2 positions are asymmetric, the S2 position is the more effective. Response dynamics. In 12 of the 16 units, excitatory



obtained in both the S1 and S2 positions, 2 position being more effective in 13 units. Onset amplitudes were determined by comparing the average discharge rate during the 1st sec of T3 with the rate during T1. The S2 and S1 responses were, respectively, 1.5 and  $1.9 \pm 0.6$  spikes/sec.

## DISCUSSION

### Response of canal neurons to linear accelerations

Semicircular canals are capable of responding to linear accelerations. Our results indicate, however, that in the monkey the response is artificial, largely the result of thermal gradients induced during exposure of the vestibular system and the petrous pyramid. The measured thermal gradients are of the correct sign to explain the directional selectivity of the response of vertical-canal neurons. We need only assume, in agreement with observation, that the lateral aspect of each canal is colder than more medial parts. The situation is less clear for the horizontal canal which, in our preparation, is least exposed of the three canals. Possibly for this reason, the directional rules were most variable for horizontal-canal neurons and these units are in general characterized by smaller responses. Our observations do not eliminate the possibility of a small response in the absence of thermal gradients. The results illustrated in Figure 1 are based on thermal measurements made external to the labyrinth and these, at best, can provide only an indirect indication of labyrinth temperatures. Critical observations would require the implantation of two thermistors within a single canal and this would have to be accomplished without perturbing the circulation of endolymph. Further, the calculations of Fettiplace et al (1968) would indicate that labyrinth temperatures would have to be monitored with an accuracy of  $0.01-0.05^\circ\text{C}$ . Thermal gradients would seem an unlikely cause for the responses observed in cold-blooded preparations by Ledoux (1949) and Lowenstein (1972). But here another explanation is possible.

It involves the supposition of a density difference between the cupula and endolymph. Both Ledoux (1949) and Lowenstein (1972) used isolated preparations. After death there may be an equilibration of the ionic compositions of perilymph and endolymph and this would cause the endolymph to become less dense than normal. Interestingly, Lowenstein's (1972) results in the ray could be explained were the endolymph more buoyant than the cupula. It must be emphasized that the required density differences are small. In the squirrel monkey, for example, calculations indicate that a difference of  $10^{-4}$  gm/cm<sup>3</sup> would produce in a canal afferent an average response of 10 spikes/sec to a 1-g linear acceleration.<sup>1</sup> Such small density differences could easily arise from experimental perturbations. Indeed the differences are so small that the presence of a response in any of the preparations currently used to record vestibular-nerve activity should not, in our opinion, be taken as convincing evidence that a similar response would occur under more normal physiological conditions.

Despite these uncertainties, the fact that the canals are potentially sensitive to such forces has both clinical and experimental implications. First, a small and unpredictable positional nystagmus is commonly observed in seemingly normal subjects (Barber & Wright, 1973; Miyata & Igarashi, 1973). The etiology of the nystagmus is undoubtedly complex, but small density differences between the cupula and endolymph may be contributory. Second, such

<sup>1</sup> The calculation is as follows. There is a linear relation  $P = K_D \alpha$ , between a constant angular acceleration  $\alpha$  and the resulting pressure differential across the cupula.  $P = K_D \alpha$  can be deduced from canal dimensions (Oman & Young, 1972). Suppose the canal were sensitive to linear accelerations because of a density difference  $\Delta \rho$  between the cupula and endolymph. Equating the pressure differentials arising from an angular and a 1-g linear acceleration gives  $\Delta \rho = K_D \alpha / gh$ ,  $h$  being the width of the cupula. The response  $s$  of a representative canal afferent to the linear acceleration is  $s = K_A \alpha$ .  $K_A$  is the afferent's sensitivity to angular accelerations. Hence  $\Delta \rho = K_D s / K_A gh$ . Typical values are  $K_D = 5 \times 10^{-4}$  dyne/cm<sup>2</sup> per deg/sec<sup>2</sup> and  $h = 2.5 \times 10^{-3}$  cm (Igarashi, 1966); also  $K_A = 2$  spikes/sec per degree/sec<sup>2</sup> (Goldberg & Fernandez, 1971). The proportionality constant between  $\Delta \rho$  and  $s$  is found to be near  $10^{-4}$  g/cm<sup>3</sup> per spikes/sec.

density differences are implicated in the larger positional nystagmus that occurs following drug intoxication (Money & Myles, 1974). And third, in studies of central vestibular neurons, it is usually assumed that units responding to static tilts receive an otolith input. The most common pattern of convergence described in the vestibular nuclei involves individual neurons receiving a canal and a presumed otolith input (Duensing & Schaefer, 1959, Markham, 1972). The conclusion is based on the observation that the same neuron responds to angular accelerations and slow head tilts. Both the conclusion, and the assumption upon which it is based, may be unwarranted.

#### *Response of otolith neurons to angular accelerations*

Our methods for categorizing peripheral vestibular neurons depend on the assumption that otolith neurons do not respond to angular accelerations. That this assumption is correct, and is not simply based on an arbitrary classification scheme, is indicated by several observations. First, virtually all units responsive to natural vestibular stimulation can easily be classified. Second, units responding to angular accelerations only do so when the appropriate canal plane has a component in the plane of motion. It is unlikely that otolith neurons, assuming that they were responsive to angular accelerations, would exhibit directional selectivities precisely correlated with individual canal planes. Third, otolith units are most commonly encountered when the recording electrode is aimed for the posterior part of the superior vestibular nerve or the ventral part of the inferior vestibular nerve. Anatomical studies (Gacek, 1969, Sando et al., 1972) demonstrate that utricular afferents are concentrated in the former region, saccular afferents in the latter region. Fourth, canal and otolith neurons can be independently distinguished on the basis of their response dynamics. Canal neurons have slow dynamics (Fig. 3), characterized by a first order time constant of about 5 sec (Fernandez & Goldberg, 1971). Otolith neurons have rapid dynamics (Fig. 5E) and have a corresponding time constant esti-

mated to be on the order of 0.01-0.1 sec (Goldberg & Fernandez, 1974).

It is not entirely clear why otolith neurons do not respond to even intense angular accelerations. *A priori* it would seem reasonable to propose that such accelerations would cause torsional movement of the otolithic membrane. Conceivably the torsional rigidity of the membrane might be high. Alternately the fast response may reflect the geometric arrangement of the hair cells. Given the morphological position of the maculae (Lindeman, 1969), head movements would deflect most sensory bundles in a direction perpendicular to the polarization axes. Presumably such deflections are relatively ineffective.

#### *Response of neurons innervating the utricle*

Canal plugging, consistent with behavioral observations (Money & Scott, 1962), abolishes the response of canal neurons to angular accelerations without having an obvious effect on activity. The procedure does not, however, prevent the canals from responding to linear acceleration. The responses cannot be explained by the presence of thermal gradients. A caloric response to angular accelerations, the circulation of endolymph and this is prevented by the plugging procedure. In another way, an occlusion of the meniscus which abolishes responses to linear accelerations should also eliminate caloric responses. Further, caloric responses are bidirectional. That is, if linear accelerations in one direction are excitatory, those in the opposite direction should be inhibitory. In our responses, though asymmetric, were bidirectional. The unidirectionality would seem to rule out any simple mechanism based on a difference in density between the cupula and endolymph.

These observations are relevant to theories of the persistent component of barbeque nystagmus. Correia & Money (1970) plugged the canals in cats and observed only a slight reduction in the nystagmus. It was concluded that the persistent component could be maintained

otolith activation. The conclusion was based on the assumption, at variance with present results, that plugging totally inactivates canals. Benson & Bodin (1966) and Steer (1968) attempted to explain the persistent component by arguing that linear forces deform the membranous canal and thereby produce a pressure differential across the cupula. In an intact canal, a constant force will be ineffective since the pressure on the two sides of the cupula will equalize within a few msec. A rotating force will, however, lead by a roller pump action to sustained deflection. Also, a constant force applied on a plugged canal might be effective, but occlusion of the membranous duct would prevent pressure equalization. Whether this or a different mechanism underlies the unidirectional asymmetric responses obtained from plugged canals cannot be decided. Of interest, though, is the fact that Benson et al. (1970), in their study of the influence of rotating linear accelerations on central neurons, found responses which were unidirectional and asymmetric.

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## ZUSAMMENFASSUNG

Einige Neuronen, die den Bogengang innervieren, reagieren auf gleichbleibende lineare Beschleunigung mit einer Es wird gezeigt, dass diese Reizbeantwortung in einem Präparat künstlich ist, und dass sie aus dem Asymmetrischen Gradienten entsteht, die auf die chirurgische Beschleunigung zurückzuführen sind. Otolithneuronen sind durch starke Winkelbeschleunigung nicht erregbar. Tamponieren des Bogengangs löscht die Reizbeantwortung der entsprechenden Affferenzen auf die Beschleunigung, ohne dass eine deutliche Wirkung auf die Ruhetätigkeit hat. Das Verfahren löscht die nicht die Reizbeantwortung des Bogengangs auf Beschleunigung. Anders als bei unoperierten Gängen wird diese Beantwortung nicht von den Asymmetrischen Gradienten veranlasst und konnte mit dem Auschluss der beherrschenden Komponente des 'bar Nystagmus' zusammenhängen.

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## ANALYSIS OF CURVES Na-24 EFFLUX FROM MEMBRANE LABYRINTH CONTAINING STRIA VASCULARIS

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The efflux of Na 24 was investigated in isolated stria from a membrane section containing stria and spiral ligament in artificial endolymph. The efflux curves were calculated and compared. Analyses were made. The theoretical possibility of exchange of sodium between compartments in different ways was considered. The calculated rate constant of transmembrane sodium fluxes  $282 \pm 0.0052 \text{ min}^{-1}$  and the half time of the exchange of sodium between intracellular compartments is 4.53 min. The distribution of sodium in tissue commonly applied simplification gives 90% Na<sup>+</sup> extracellular compartment and 3% Na<sup>+</sup> in the lular compartment of the total tissue sodium.

The possibility of measuring the rate constant of membrane electrolyte fluxes in this tissue seems promising for future investigation of the mechanism by which they are transported.

Variability of the electrolyte composition of artificial endolymph as an extracellular fluid, of potential significance (Békésy, 1952) are cause of much speculation. Investigators obtained a great deal of experimental data concerning these phenomena in various experimental conditions (Konishi et al., 1966, Kuypers et al., 1970, Prazma, 1969, Sellick & John, 1972, Silverstein, 1970, Tanaka & Brown,

as well known that the distribution of electrolytes between adjoining spaces, and the kinetics of their exchange, are the basis on which the real phenomena are explained (Hodgkin,

So far, however, we have had no kinetics about the exchange of electrolytes between stria vascularis cells and endolymph.

The present work attempts to estimate some kinetic parameters of sodium transport, on the basis of efflux curves.

### METHOD

The experiments were carried out on an isolated section of a guinea pig membrane labyrinth which contained stria vascularis and spiral ligament. The animals were decapitated, the skulls quickly opened and the cochlea taken out. In artificial endolymph (4°C) a part of the membrane labyrinth which contained stria vascularis and spiral ligament was then isolated. The whole procedure of preparation was done using a stereoscopic microscope (12×) and lasted about 20 min. Subsequently the tissue was incubated in a medium similar to endolymph (artificial endolymph) which contained 144 mM K<sup>+</sup>, 5 mM Na<sup>+</sup>, 3 mM Ca<sup>++</sup>, 2 mM Mg<sup>++</sup>, 135 mM Cl<sup>-</sup>, 15 mM HCO<sub>3</sub><sup>-</sup>, 12 mM H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, and 6 mM glucose, at a temperature of 37.5°C and pH 7.35. The total volume of incubation medium was 3.0 ml and it was circulated by a constant stream of mixture (97% O<sub>2</sub>, 3% CO<sub>2</sub>). The incubation medium contained Na 24, specific activity was mCi/ml. After the incubation, which lasted about 40 min, the preparation was rinsed (Mullins & Frumento, 1963) in a wash-out solution, also artificial endolymph of exactly the same composition, but without isotopes. In this way, samples were obtained with Na-24 which ha

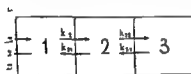


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intuitive expression of kinetics of sodium nge at the cell membrane level (Zierler, Van Liew, 1967) Furthermore, if we are empt to combine the kinetics of sodium nge between stria vascularis cells and endo- with resting endolymphatic potential, we bear in mind several possible means of separate compartments obtained from mental three-component curves

Consider for example the following cases the work of Levin & Patlak (1972) The three-compartment model series in which ree compartments are connected together



values of the particular rate constants exchange between compartments are given by

$$k_{01} = \frac{\sum_{i=1}^3 B_i \lambda_i}{\sum_{i=1}^3 B_i \lambda_i^2} \left[ \frac{\sum_{i=1}^3 B_i \lambda_i^3}{\sum_{i=1}^3 B_i \lambda_i^2} - k_{12} - k_{21} \right]$$

$$k_{12} = \frac{1}{k_{21}} \left[ \frac{\sum_{i=1}^3 B_i \lambda_i^4}{\sum_{i=1}^3 B_i \lambda_i^3} - k_{11} \right]$$

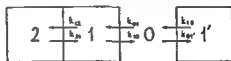
$$k_{21} = \frac{1}{k_{12} k_{23}} \left[ \frac{\sum_{i=1}^3 B_i \lambda_i^5}{\sum_{i=1}^3 B_i \lambda_i^4} - k_{11} - 2k_{21} k_{12} k_{23} \right]$$

$$k_{23} = k_{21}$$

$$k_{22} = k_{23} k_{12} k_{21} \left[ \frac{\sum_{i=1}^3 B_i \lambda_i^6}{\sum_{i=1}^3 B_i \lambda_i^5} - k_{11}^2 - k_{12} k_{21} \right] \times (3k_{11}^2 + 2k_{11} k_{22} + k_{12} k_{23} + k_{22}^2)$$

As described by Levin & Patlak (1972), in the flow from medium to tissue, coefficient  $B_1$  is negative. The efflux from tissue to medium is described by the same values ( $B_i$ ), except that they are positive. Thus in the equation for  $k_{01}$ , the sign is plus.

One should note that the calculated values for  $k_{ij}$  here describe the kinetics of exchange but we cannot consider their values as a ratio constant of the efflux between cellular space and medium. The reason for this divergence is assumed by considering the model equality of compartments. It is possible to obtain more adequate values for  $k_{ij}$  by taking into consideration the size of the compartments. A second possibility is the coexistence of two compartments in series and one in parallel.



The particular values of rate constant exchange between the compartments are given

$$k_{01} = (B_1 \lambda_1 + B_2 \lambda_2)$$

$$k_{10} = \frac{B_1 \lambda_1^2 + B_2 \lambda_2^2}{B_1 \lambda_1 + B_2 \lambda_2}$$

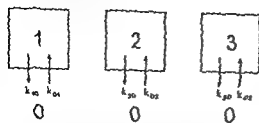
$$k_{11} = \frac{B_1 \lambda_1^3 + B_2 \lambda_2^3}{B_1 \lambda_1^2 + B_2 \lambda_2^2}$$

$$k_{12} = \frac{B_1 B_2 \lambda_1 \lambda_2 (\lambda_1 - \lambda_2)^2}{B_1 (\lambda_1 + B_2 \lambda_2) (B_1 \lambda_1^2 + B_2 \lambda_2^2)}$$

$$k_{21} = \frac{(B_1 \lambda_1 + B_2 \lambda_2) \lambda_1 \lambda_2}{B_1 \lambda_1^2 + B_2 \lambda_2^2}$$

$$k_{20} = \lambda_2 \quad k_{01'} = B_2 \lambda_2$$

A third possibility is a three-compartment model directly connected with medium



The values of  $k_{10}$ ,  $k_{20}$ ,  $k_{30}$ , are given immediately by  $\lambda_1$ ,  $\lambda_2$  and  $\lambda_3$ , though  $k_{01}$ ,  $k_{02}$ ,  $k_{03}$  are given by  $B_1\lambda_1$ ,  $B_2\lambda_2$ ,  $B_3\lambda_3$ . In this case the rate constant for each particular compartment is equal to the exchange constants  $k_{0i}$  of the cellular membrane. The above model may also be an approximation in the case of three kinds of cells which have differing kinetics of exchange, assuming that the diffusion process in extracellular space is very quick. Another possible interpretation of this model is that one compartment is extracellular space, when geometry and other conditions are the cause of small exchange between cells and extracellular space.

The fourth possibility is thus: compartment 1 is the extracellular space and compartments 2 and 3 represent two different kinds of cells (Fig. 5). One can see that the exchange between these cells and the medium is possible only via the extracellular space. Mathematically this model may be described by equations

$$\frac{dC_1}{dt} = k_{01}C_0 + k_{21}C_2 + k_{31}C_3 - (k_{10} + k_{12} + k_{13})C_1$$

$$\frac{dC_2}{dt} = k_{12}C_1 - k_{21}C_2$$

$$\frac{dC_3}{dt} = k_{13}C_1 - k_{31}C_3$$

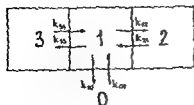


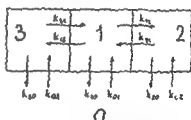
Fig. 5

The general solution of the above system of equations has the form

$$\frac{C_1 + C_2 + C_3}{C_0} = B_0 + B_1 \exp(-\lambda_1 t) + B_2 \exp(-\lambda_2 t) + B_3 \exp(-\lambda_3 t)$$

where  $C_0$ ,  $C_1$ ,  $C_2$ ,  $C_3$  represent the sodium concentration in the compartments and  $C_0$  medium. Following Levin & Patlak (1967) the same expression was obtained for  $k_{0i}$ , though for the next coefficient other relations were obtained which do not give a solution for  $k_{ij}$ .

A more complicated but more probable solution is given below



Mathematically this model describes a system for the differential equations

$$\frac{dC_1}{dt} = k_{01}C_0 + k_{21}C_2 + k_{31}C_3 - (k_{10} + k_{12} + k_{13})C_1$$

$$\frac{dC_2}{dt} = k_{02}C_0 + k_{12}C_1 - (k_{20} + k_{21})C_2$$

$$\frac{dC_3}{dt} = k_{03}C_0 + k_{13}C_1 - (k_{30} + k_{31})C_3$$

The solution of these equations is more complicated than the one before last.

Irrespective of the theoretical possibilities presented above in exact terms of the particular rate constant of exchange between particular compartments, one first approximation assumes that the rate constants  $\lambda_1$ ,  $\lambda_2$  concern the efflux of cellular sodium and the slow rate constant  $\lambda_3$  represents the transmembrane fluxes of sodium (Brading & Jones, 1969; Brading, 1971; Strom, 1973). In such an interpretation, investigated tissue about 90% of  $\text{Na}^{24}$  is buried in extracellular space and 10% is

ular Taking into account Huxley's correction factor (Huxley, 1960) multiplied by Brading (1971) and Wahlström the intracellular compartment contains 3% Na of the total tissue content, the 7% is probably very rapidly exchangeable sodium located on the surface of the tissue. The value of the rate constant of transanal effluxes is equal  $0.0282 \text{ min}^{-1}$  in endolymph and is similar to that obtained by Wahlström (1973) from rat portal vein— $0.0280 \text{ min}^{-1}$  in Krebs solution, and by Brading (1971) in *aemia coli* in Krebs solution. This agrees, puzzling because it exists in very different media. It would appear that some explanation concerning the mechanism of electrolyte transport in stria vascularis cells and endolymph give more precise measurements of transanal effluxes in various experimental conditions, even if at the outset we were to assume a procedure similar to that used in morphologically homogeneous tissue.

## ZUSAMMENFASSUNG

In künstlichen Endolymphen-Milieu wurde die Ausbreitung des Na 24 aus dem die Stria vascularis und das Labyrinth enthaltenden Teil der Membran untersucht. Es wurden gewisse theoretische Aussagen des Natriumaustausches zwischen den verschiedenen Teilen diskutiert. Der berechnete Wert der Diffusionskonstante der Natriumauswanderung aus den untersuchten Geweben beträgt  $0.0282 \pm 0.0005 \text{ min}^{-1}$ . Die Halbwertszeit des Natriumaustausches im intrazellulären Teil beträgt 33 min. Der geschätzte Natriumabbau im Gewebe gibt bei den für gewöhnlich angewandten Vereinfachungen 90% im extrazellulären Teil lokalisiertes Natrium und ca. 3% Gesamt-Natrium im intrazellulären Teil. Die Möglichkeit der Messung der Geschwindigkeitskonstante des transanal Elektrolytenstromes in diesem Gewebe sowie neue Forschungsmöglichkeiten für den Austausch von Elektrolyten zwischen den Zellen dieses Labyrinthes und der Endolymphen.

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## SURGICAL ANATOMY OF THE GENICULATE GANGLION

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**Abstract** The position and the average dimensions of the geniculate ganglion have been measured with the Zeiss screw micrometer in 24 temporal bones obtained from the histological collection of the ENT department of the University of Zurich. The geniculate ganglion has been found to lie like a cup over the anterior portion of the genu of the VII nerve. Its dimensions have shown only minimal variations (standard deviation of average length, height and width less than 16%). Surgical resection of the anterior third of the genu of VII nerve has been shown to include 90% of the ganglion cells but not to endanger the motor fibres. These surgical measurements are the basis for performing a resection of the geniculate ganglion in cases of geniculate neuralgia.

The geniculate ganglion is a complex system containing ganglion cells of the sensory (taste) fibres of the greater superficial petrosal nerve and corda tympani as well as the preganglionic secretory fibres for the sphenopalatine ganglion. According to Kure & Sano (1936) the geniculate ganglion also contains other sensory ganglion cells which are not related to the function of taste but are believed to be responsible for the pain occurring in petrosal neuralgia. Clinical experience (Fisch, 1973) has shown that excision of the geniculate ganglion gives better long term results in the relief of geniculate neuralgia than does dissection or excision of the greater superficial petrosal nerve. The present study was undertaken with the aim of defining the exact topographical position of the geniculate ganglion in order to permit the surgical excision of this structure without damaging the neighbouring motor fibres of the facial nerve.

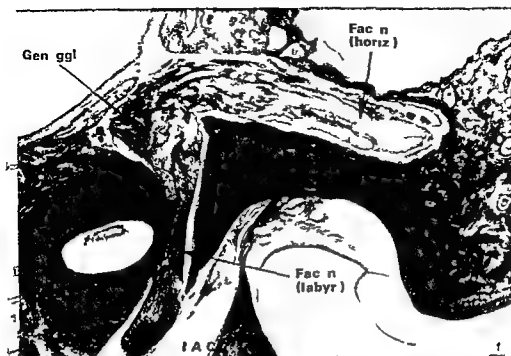
### MATERIAL AND METHOD

The topography of the geniculate ganglion was established by measurements obtained from histological sections of the temporal bones of the ENT-department of the University of Zurich. Twenty-four temporal bones belonging to patients ranging from 11 to 82 years were examined. The temporal bones were cut in a horizontal plane in a vertical plane. The average thickness of the celloidin-embedded section was 10 µm. The measurements were performed with a Zeiss screw micrometer ocular.

### RESULTS

In all examined specimens the geniculate ganglion cells were found lying like a cup over the anterior portion of the external genu of the facial nerve (Figs 1 and 2). Only a few scattered ganglion cells were found medial to the genu along the labyrinthine meatal portion of VII nerve. In 24 specimens (i.e. 20% of the cases) the anterior part of the ganglion was lying free of the facial nerve, directly under the dura of the cranial fossa.

The average dimensions of the geniculate ganglion are shown in Fig 3. In the horizontal plane the ganglion is triangular in shape with an average length of 1.09 mm (Fig 3a). A section through the ganglion shows the



Labyrinthine and horizontal portion of the facial nerve (H.E. 10)

Genus of the facial nerve Gen ggl - geniculate ganglion Fac n - facial nerve (labyr) - labyrinthine part

of the facial nerve (horiz.) horizontal part of the facial nerve IAC - internal auditory canal GSPN - greater superficial petrosal nerve (H.E. 60)

average width of this structure is 0.76 mm with an average height of 0.6 to 0.8 mm. The posterior and most proximal part of the ganglion overlaps for a short extent the motor fibres (Fig 3b). This overlapping portion of the ganglion is, however, so thin that it can be neglected for surgical purposes. This is particularly true in view of the fact that according to the investigations of Kure & Sano (1936) the most important sensible ganglion cells are concentrated in the anterior portion of the ganglion just before the origin of the greater superficial petrosal nerve.

According to the measurements presented (Fig 4) surgical resection of the frontal third of the facialis genu permits excision of the majority of the ganglion cells without any danger to the motor fibres. In many instances the line of division between geniculate ganglion cells and motor fibres can be identified using the operating microscope. In those instances where this division is not visible, resection of the anterior third of the genu of the VII nerve permits excision of more than 90% of the ganglion cells including all those which are probably responsible for the pain related to geniculate neuralgia.

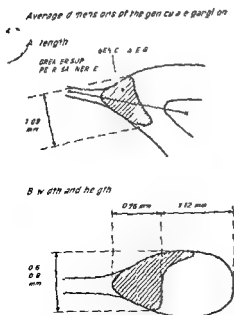


Fig 3

## The surgical excision of the geniculate ganglion

## Anatomical measurements

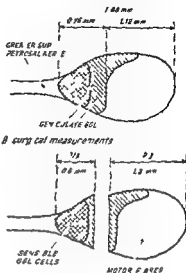


Fig 4

## CONCLUSIONS

Histological measurements of the geniculate ganglion have shown that this structure is the frontal third of the genu of the VII nerve. The dimensions of the ganglion are only slightly variable since the standard deviation of the average length, width and height of the structures was in all instances less than 10%. For surgical purposes, 90% of the geniculate ganglion cells of the geniculate ganglion can be excised by removing the anterior third of the genu.

## RÉSUMÉ

La position et les dimensions moyennes du ganglion géniculé ont été mesurées sur 24 rochers. Le ganglion se présente en forme de coupe au-dessus de la partie antérieure du genou du nerf facial. Ses dimensions n'ont montré que des variations minimales (écart standard de la longueur, de la largeur et de la hauteur inférieure à 10%). Une section chirurgicale du tiers antérieur du genou du nerf facial comprend des cellules ganglionnaires et évite tout dommage aux fibres motrices. Ces mesures constituent la base pour la résection du ganglion géniculé dans les névralgies géniculées.

## ZUSAMMENFASSUNG

Anhand von 24 histologisch verarbeiteten Felsen aus der Sammlung der Hals-Nasen-Ohren-...

sität Zurich wurde die Topographie des Ganglion geniculi untersucht. Ziel der Studie war, Lage und Ausdehnung der Ganglienzellen im Bereich des Kniees zu lokalisieren. Das Ganglion geniculi sitzt auf dem Facialisknäuel. Dabei zeigen Form und Ausdehnung des Ganglions im Vergleich zum Gesamtknie des Nerven von Fall zu Fall nur geringe Unterschiede (unter 10% in allen Richtungen). Eine partielle Resektion des ventralen Drittels des Kniees gewährleistet aufgrund der vorliegenden Untersuchung die Entfernung von ca. 90% der Ganglienzellen ohne Gefährdung der motorischen Facialisfasern. Das Ergebnis dieser Verhältnisse bildet die Grundlage zur chirurgischen Behandlung von Neuralgien des Ganglion geniculi.

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## AN INTERACTIVE PROGRAM FOR THE ANALYSIS OF ENG TRACINGS

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**Abstract** An interactive program for clinical and research evaluation of vestibular nystagmus has been developed. Because of the interactive structure of the program, the clinician can make immediate decisions on the interpretation of controversial aspects of the nystagmogram, therefore even the more irregular and noisy ENG tracings, frequently obtained from pathological subjects, can be easily processed. In this way, speed and accuracy of computer processing are coupled with intelligent activity and critical judgement of the clinical evaluation. The program can be implemented on a general purpose minicomputer and no specific technical training is required for its use. The structure of the program and the operations performed by main subsystems are discussed in detail. Examples of ENG tracings processed with the program are reported.

The analysis of vestibular nystagmus recorded by electronystagmography (ENG) is becoming the preferred procedure for the valuation of vestibular function in many clinical and research investigations.

However, direct manual measurements of various elements of the nystagmic response, such as number of frequency of nystagmus beats, total angular displacement of eyes (Gesamt-amplitude), slow phase angular velocity, are very cumbersome and time-consuming.

For this reason several analog and computer methods have been proposed for automatic or semi-automatic analysis of ENG records: both methods have advantages and disadvantages.

Analog devices (Henriksson, 1955, Benson & Stuart, 1966, Guedry & Turnipseed, 1968, Voots, 1969, Blauert, 1972, Max et al., 1973, McClure et al., 1973) are relatively unexpensive

and easy to use, but they are scarcely accurate: their analysis is limited to single elements of the nystagmic response and may give rise to errors due to integration of noise from various sources or to lack in distinguishing nystagmic from random eye movements or artifacts.

Digitized data processing systems, although more complex and expensive and require more computer facilities and well trained operators, on the other hand they give a detailed analysis of the various elements of the nystagmic response, can automatically recognize specific nystagmic characteristics and eliminate biological or electronic artifacts, finally can be more easily modified in relation to specific requests of the clinician.

In recent years many authors (Herbert, 1968, Matz et al., 1970, Loth et al., 1971, & Young, 1971, Anzaldi et al., 1972, 1973, Nagle & Anderson, 1972, Omer, 1973, Schuider et al., 1973, Ward, 1973) have developed several programs for the analysis of nystagmus which perform this operation in a fully automatic way. This kind of program gives excellent results if applied to selected records, presenting an even and regular sequence of beats with well defined slow and fast phases and rarely interrupted by saccades. This is the case of optokinetic nystagmus, vestibular nystagmus from normal subjects submitted to particular stimulations (e.g. prolonged constant accelerations, sinusoidal tests) for research purposes. On the contrary, for ENG tracings obtained from caloric or rotational tests performed in clinical setting, es-

hological subjects, are often very irregular and their analysis is frequently difficult even for a well trained clinician. Care must be taken in ignoring uncontrolled artifacts or noise and in removing sources, in detecting the beginning and the end of the nystagmic response or the direction of the nystagmus direction, in identifying, in recognizing and classifying peculiar aspects of the tracing which may have some diagnostic importance (pauses, dysrhythmias, square waves, irregular movements, etc.) (Jongkees, 1973).

One of the programs so far developed can automatically detect these interpretation problems and this is the primary reason for limiting the computer analysis of nystagmus in clinical practice. In fact, automatic processing of ENG tracings can be performed only by using very complex programs, based on the more sophisticated methods of pattern recognition.

An alternative solution is offered by an interactive approach (Kamal, 1973): these systems employ computational algorithms for detecting, recognizing and classifying nystagmus beats but, because of anomalies or irregularities of the tracings, they leave to the operator the task of their interpretation. In this way we have a very useful instrument, in which the computer and the accuracy of computer analysis are supplemented with intelligent activity and critical judgment of the clinician (Anzaldi et al., 1973). This paper describes an interactive program for analysis of nystagmus (TAIS) recently developed at the Centro per l'Interazione Operatore-Macchina and now currently in use at the Otorinolaringoiatrica Clinic of the University of Turin.

The interactive pattern analysis system can operate in line with data acquisition, saving a lot of the time required by the operator in analyzing and correlating the clinical data, comparing different samples of the ENG tracings for making decisions in order to process single tracts of the nystagmogram on the basis of informations previously acquired. We therefore developed an off-line system consisting of different phases: vestibular examination and recording of vestibular nystagmus,

both on paper and on magnetic tape, analog-to-digital conversion and storing of the data in the mass-memory of the computer, visualization of the nystagmogram on the computer display and its analysis, evaluation and printout of the results.

Both for clinical and research purposes we perform different vestibular tests: caloric (Fitzgerald-Hallpike, Valsalva) or rotational (post-rotational or pre-rotational tests with different velocity profiles).

Ocular movements in the horizontal plane are recorded by means of two bitemporal silver-silver chloride skin electrodes (Beckman type 3503), with a ground electrode placed on the forehead. The electrodes are connected with a d.c. amplifier (Beckman Dynograph Type R) and the stimulation and nystagmus signals are displayed simultaneously on a polygraph: the output of the amplifier is also coupled with a four-channel FM tape recorder (Ampex FR 500). One of these channels is used for recording a control signal which marks the beginning and the end of nystagmus registration and can be utilized in the subsequent A/D conversion for synchronizing purposes; another channel is used for recording the duration of the stimulus in caloric tests or the chair velocity in rotational tests.

In electro-oculography the relationship between ocular displacement ( $\theta$ ) and the variation of the corneoretinal potential derived by the electrodes ( $V$ ) is linear up to  $30^\circ$  to each side of fixation; therefore, for correct recording of larger eye movements, which are often present at the beginning of post-rotational nystagmus, we must construct the function  $V = f(\theta)$ . For this reason, each vestibular stimulation is preceded by a complex calibration procedure, requiring a series of ocular displacements of different amplitude ( $\pm 10^\circ$ ,  $\pm 20^\circ$ ,  $\pm 30^\circ$ ,  $\pm 40^\circ$ ). Processing of nystagmographic data is performed by a general purpose minicomputer (Laben 70, with 16K, 16-bit words and 1.33  $\mu$ sec. cycle time), equipped for the analog-to-digital conversion and for the graphic presentation of the output (display-terminal Tektronix 4010).

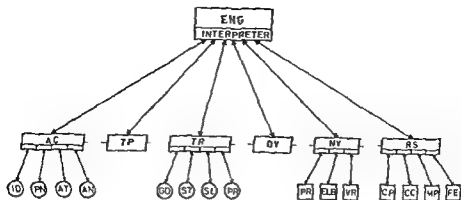


Fig 1 General structure of the program TAIS

The program for the analysis of nystagmus is written in FORTRAN IV and, for input-output operations in Assembler. As shown in the block diagram of Fig 1, the program (TAIS) has a tree structure, composed of a main program and several subsystems, in which every branch can be activated at operator request.

AC data acquisition and retrieval,  
 TR analysis of calibration tracing  
 NY analysis of nystagmus tracing,  
 TP magnetic tape drivers  
 DY graphic input-output drivers,  
 RS results presentation

On the basis of the operator's requests, the main program activates a selected subsystem which, in turn, activates a series of routines, each performing a specific task.

In the following pages we will give a description of the main subsystems, omitting those, such as TP and DY, which are only service subsystems.

AC: analog nystagmographic data, recorded on magnetic tape, are digitized and stored in mass memory (9 Track magnetic tape) computer. The highest frequency in the spectrum of vestibular nystagmus rarely exceeds 20 Hz and therefore the signal is anti-aliased and filtered at 25 Hz. The sampling rate is 100 samples/sec. In order to follow the digitized data, graphic tracing is coupled with various information accepted as additional inputs by the program (e.g. patient data, data and number of examinations, type of stimulation, other references needed for the otoneurological diagnosis).

TR: this program performs the calibration tracing and calculates the

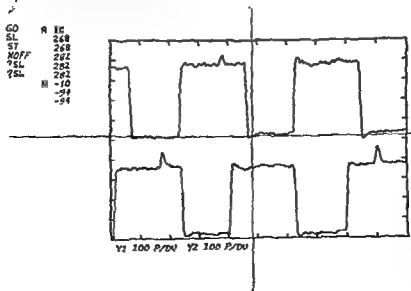


Fig 2 Measurement of levels corresponding to the stimulation of  $\pm 10^\circ$  degrees.

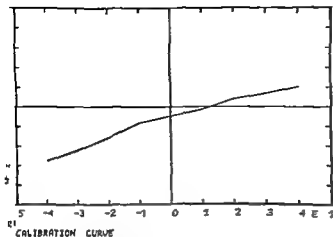


Fig 3 Display presentation of the calibration curve

$V = V(\theta)$  Calibration eye movements, especially in pathological subjects, are often irregular and their anomalies may have important diagnostic significance (Haring & Sons, 1973) for these reasons, in our interactive program the analysis of these movements and the measure of potential differences depending to eye displacements are tasks assigned to the human operator. Calibration is presented step-by-step on the computer display and the clinician marks the different initial levels using a graphic cursor (Fig 2) values so measured on the vertical axis are memorized and, at the end of this phase, the calibration curve is presented on the display

(Fig 3). Moreover, if non linearities are present in the calibration, they are automatically corrected during the subsequent analysis of the nystagmus.

**NY** This is the specific program for analysis of ENG tracing. The tracing is presented step-by-step on the display, on the left side of the display is also presented a menu of the routines which can be activated by the operator. This operation is performed by typing the code of the selected routine in response to the question mark presented by the computer. So, the operator can detect and mark, using the graphic cursor, the beginning (BG) and the end (ST) of the response, the transition from the first to the

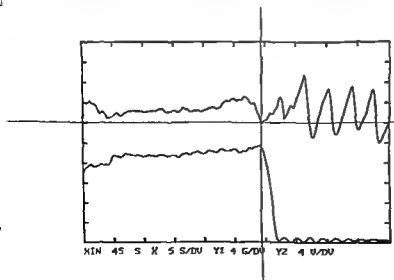
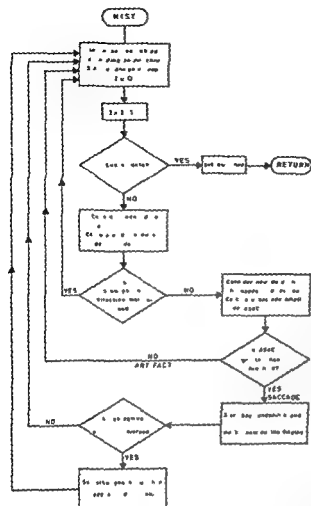


Fig 4 Determination of the beginning of a post-rotational nystagmic response. The lower trace shows the chair velocity.



**Fig. 5** Flow chart of the algorithm NIST

second phase (F1-12) of nystagmus in a post-rotational test or the reversal of nystagmus direction in the different periods of a sinusoidal

test (Fig. 4) The lower trace of the presents the stimulation signal (VD) (Fig. 4) at operator request (VH), the following of the nystagmus record (Fig. 6)

During this phase of processing the system can detect the artifacts present in the tracing these artifacts can be manipulated (IN) or, if the clinician attributes some diagnostic significance, can be stored (CM) and retrieved.

In the next phase of elaboration, the program reactivates the routine for automatically tracing nystagmus beats (GO). The program composing this routine (NIST, Fig 5) is described in detail elsewhere (Anzaldi & 1972) on the basis of the nystagmus previously defined by the operator, the program recognizes the first slow phase eye movement at every point of the input signal in comparison with the preceding and the difference is calculated and stored. Whenever a reversal in the direction of eye movement occurs, the program distinguishes between a fast phase or a slow phase eye movement. If this reversal is observed in more consecutive samples and if its amplitude exceeds a threshold value, the program recognizes that a saccade has occurred. The threshold (usually of 1 or 2 deg) is prefixed by the operator activating the routine SG and may be modified on the basis of the characteristics of the tracing under examination. At the end of the analysis, nystagmus beats so detected are

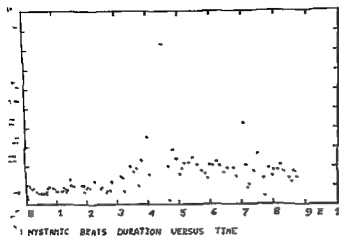


Fig 7. Display presentation of the time course of the duration of nystagmus beats in a post rotational test

ally marked on the display by a series of vertical bars (Fig 6). In this way the operator can recheck the validity of the recognizing operation performed by the computer and make corrections, e.g. including (AD) or excluding (DL) single beats or re-examining a part of the ENG record using different threshold values (RP).

The output of the subsystem NY is a synthetic description of the nystagmic response, each nystagmus beat being defined only by its end position. The response coded in this way is punched on paper tape together with the data for the identification of ENG tracing, clinical information and operator comments. The punched data can be retrieved and used for further elaborations of nystagmus (statistical analysis, correlation

coefficients, frequency histograms, etc.), all performed automatically by the computer.

RS, this subsystem, operating on the output of the subsystem NY, gives automatically the values of the conventional elements of the nystagmic response, i.e. duration, number of beats, total eye displacement (Gesamt amplitude), and a running time display of the duration (Fig 7), amplitude and frequency of nystagmus beats. RS can also perform a more complex elaboration of nystagmus calculating cumulative slow phase eye position (Fig 8) and slow phase velocity (Fig 9) (Anzaldi & Boselli, 1973).

In presence of spontaneous nystagmus, the operator can manually correct the automatic processing of the response, by typing the required instructions.

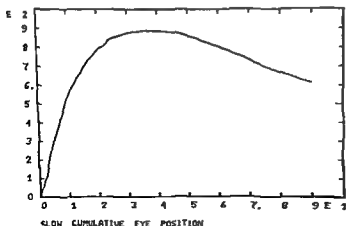


Fig 8. Display presentation of the slow cumulative eye position in a post rotational test.

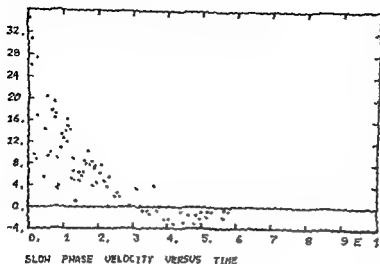


Fig. 9 Display presentation of the course of slow phase velocity in a rotational test

The program system comprises other additional subsystems (PI and PS) for automatically calculating the parameters of the vestibulo-ocular reflex of the subjects under examination, on the basis of a mathematical model proposed by Schmid (1970, 1974) these subsystems are not described in detail in this paper, being used at present only for research purposes.

TAIS interactive program has been used over two years for currently processing ENG tracings recorded at the Otorhinolaryngological Clinic of the University of Pavia: systematic comparisons show a good correspondance between the results obtained from hand-scored and automatically-scored records. In our opinion, the reliability of the program is mainly due to its interactive form, which consents a strict and continuous partnership between the clinician and the computer and therefore markedly reduces the occurrence of errors. For research purposes, our digital computing technique provides a rapid and precise method for evaluating many aspects of the nystagmic response and give much quantitative information about correlations existing between the various elements of the nystagmus: this information can be important for the understanding of the intrinsic mechanisms that produce nystagmus.

In clinical setting, TAIS interactive system is effective and reliable in processing ENG records, even in the case of irregular and noisy tracings obtained from pathological subjects, the com-

plete analysis of caloric or rotational test can be performed in about 10 minutes.

No specific technical background in the instruments or in computer usage is required to the operator, moreover the tree-structure of the program, comprising a series of subroutines with limited memory occupation, allows the use of a general purpose minicomputer. In these reasons the TAIS interactive system is especially suitable for clinics and laboratories which do not have complex and expensive computer installations.

## RÉSUMÉ

Un programme interactif pour l'analyse du nystagme vestibulaire a été développé. En raison de cette forme interactive, le programme offre au clinicien la possibilité d'intervenir directement pour l'interprétation de données claires du nystagmogramme (déterminer le début et de la fin de la réponse nystagmique, les instants d'inversion du nystagmus, éliminer les artefacts) de cette façon même les tracés les plus réguliers et compliqués, tels qu'on les obtient fréquemment dans la routine clinique à partir de sujets pathologiques, peuvent être aisément analysés. Le programme peut être implémenté sur un petit ordinateur et son utilisation ne comporte pas de connaissances techniques spécifiques de la part de l'opérateur. Dans cet article sont décrites la structure générale du programme et de ses sous-programmes principaux et sont rapportés des exemples de tracés ENG.

## ZUSAMMENFASSUNG

Es wurde ein interaktives Programm für die Analyse des vestibulären Nystagmus entwickelt. Diese Form der Interaktion ermöglicht es dem Arzt, direkt in die Interpretation der Nystagmusdaten einzugreifen. Auf diese Weise können auch komplizierte und unregelmäßige Nystagmusaufzeichnungen, wie sie häufig in der klinischen Routine von Patienten erhalten werden, leicht analysiert werden. Das Programm kann auf einem kleinen Computer installiert werden und erfordert keine speziellen Kenntnisse des Benutzers. In diesem Artikel wird die allgemeine Struktur des Programms und seiner Unterprogramme beschrieben, und es werden Beispiele für ENG-Aufzeichnungen gezeigt.

z dunkelsten Teile des Nystagmogramms zu  
ieren (Bestimmung des Anfangs und des Endes  
wort des Nystagmus oder des Augenblicks der  
ung des Nystagmus, Beseitigung der Fehler)  
nen auch die unregelmässigsten und kompli-  
Linien, die man gewöhnlich in der klinischen  
antritt, leicht analysiert werden Für dieses  
um braucht man nur einen kleinen Allzweck  
er, der keine besondere technische Kenntnisse  
erater verlangt In dieser Arbeit werden die  
ne Struktur des Programms und seine wichtigsten  
me beschrieben Es werden auch Beispiele der  
von ENG angeführt

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## LOCAL TISSUE EFFECTS OF SURFACE-APPLIED ENT DRUGS

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**Abstract** Local tissue effects caused by a selected group of topically applied ENT-drugs are analysed. By means of a testing system consisting of vital microscopy, infrared thermography and microangiography applied to hamsters and rabbits, an evaluation of the varying degree of tissue injury is made. According to the degree of tissue damage the tested substances could be graded in three groups (I-III). The drugs belonging to group I show fairly little microcirculatory disturbance, while those belonging to group III cause tissue necrosis. Our conclusion is that topically applied drugs should be used with caution, especially on previously injured tissues.

Drugs which are applied onto or into tissues may damage normal tissue as well as diseased tissue, as demonstrated in studies on local tissue effects of wound disinfectants, corticosteroids, sodium, fluorides, chymopapain, and X-ray contrast media (Brånemark, 1967, 1969, 1972, Sørensen & Nilsson, 1972).

This injury is more pronounced in tissue where the covering epithelial barrier has disintegrated or the tissue itself has a lowered threshold for injury, as in inflammatory conditions. Tissue injury is mainly mediated and manifested by the microvascular system.

For the analysis of the response of the microvascular system to topically applied drugs, an experimental system was designed. It consists of vital microscopic evaluation of drugs applied onto intact epithelium, onto experimentally denuded subepithelial structures, and injected into epithelium-covered tissue in the hamster's cheek pouch. This model enables analysis of

tissue microcirculation over 6-8 hours of the drugs as well as the restitution of the microvascular system can be evaluated. The long-term consequences for the tissue of the drug are delineated by microangiography. The dynamic events in the area of the ear drum, subperichondrally in the rabbit's ear, where the drug was deposited, can be evaluated by thermography. Macrophotographic evaluation of the ear in transillumination gives additional information on the inflammation.

The purpose of the present investigation is to elucidate the possible tissue injury by ENT drugs which are clinically applied to mucous membranes with intact or disintegrated epithelial covering.

### MATERIAL AND METHODS

The following drugs were analysed, selected as representing some common topically applied substances being used in clinical practice. It may be observed that in no drug is the pharmacological effect itself to be corrosive.

- |                                |                 |
|--------------------------------|-----------------|
| 1 Carbocain Thesat 4+1 %       | 4 Kenacort      |
| 2 Carbocain Thesat spray 5+2 % | 5 Mercurochrome |
| 3 Chloromycetin Topical        | 6 Nafazolin     |

Supported by grants from the Swedish Medical Research Council, Göteborg University, and Göteborgs Läkaresällskap.



Fig. 1. Bird's eye view of exposed cheek pouch of hamster.

1. Tobocetin	12. Terracortril
2. Tobocetin	Polymyxin B
3. Tobocetin	13. Tetracain 1%
4. Tobocetin	14. Tetracain 2%
5. Tobocetin	15. Xyllocain 4%
6. Tobocetin	16. Xyllocain spray
7. Tobocetin	10%.

#### Vital microscopy of hamster cheek pouch

**Animals and material** Hamsters of both sexes weighing about 100 g were anaesthetized with pentobarbital 30 mg/kg i.m. The animal was immobilized in a special microscope stage with body temperature maintained. The pouch was exposed over a glass plate so that a proper sensor system could be used for transillumination (Fig. 1). The cheek pouch was continuously irrigated with Tyrode's solution at constant temperature. This is important as microcirculation in the cheek pouch is sensitive to dehydration and changes in temperature. Three experimental designs were used: (1) removal of epithelium, (2) production of a defect in the pouch, and (3) deposit of material. Each substance was applied onto intact epithelium once in every experiment. In 30 experiments the pouch was everted and the epithelium and connective tissue on one side over an area approximately 2 mm in

diameter, were gently removed by means of a stereomicroscope and microsurgical instruments (Fig. 2). This procedure results in a microwound with exposure of connective tissue capillaries in the defect. The medium was applied with an eye dropper in such an amount that maximal effect of the drug was established during the application, after 10 minutes the medium was washed away with Tyrode's solution.

(3) In 64 experiments 0.05 ml of the drug was injected into the upper layer of the cheek pouch (Fig. 2), the pouch then being watched for up to 5 hours under standard environmental conditions maintained by irrigation with Tyrode's solution. The observations were made with transillumination in a modified Leitz intravital microscope (objective UO X 23, NA 0.55 and UO X 55, NA 0.84) with microphotographic recording of structures and microcinematographic registration of flow patterns (Brånemark, 1964).

Corpuscular flow velocity was measured in selected small venules by a comparative optoelectronic method (Brånemark & Jonsson, 1963).

#### B. Infrared thermography (IRT)

Twenty four rabbits of both sexes weighing approximately 1.5–2 kg were used for repeated thermographic studies of the heat emission pattern from the ears. Heat emission from rabbits ears predominantly emanates from the subcutaneous vessels. Increased or decreased circulation is reflected as a change of the surface temperature.

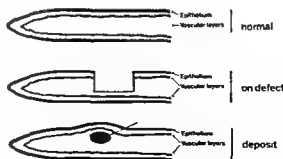


Fig. 2. Diagrammatic representation of various experimental procedures for vital microscopic evaluation of microcirculation in hamster cheek pouch.

The IRT registrations were performed with an AGA Thermovision mod 669. The instrument was calibrated against a temperature reference. The spatial resolution capacity for the actual experimental set-up allowed of separation of individual vessels with a diameter of around 0.5 mm. The thermal resolution was about 0.4°C. The room temperature was kept within  $22 \pm 1^\circ\text{C}$ . The heat emission from each ear was registered before the deposition of the substances. Registrations were then performed immediately after deposition and thereafter once a day during one week.

Three parameters were used for evaluation of the local toxic effects of the tested substances.

1 Immediate decrease of heat emission from deposit area. This parameter indicates the vasoconstrictive capacity of the deposited substance and does not necessarily parallel the definite tissue damage.

2 Development of emission pattern in the deposit area as an indication of inflammatory and healing reactions or grave tissue injury with irreversibly blocked circulation.

3 Development of emission pattern from marginal areas, not in direct contact with the deposited substance.

Each of these parameters could be graded in relation to the time factor and to the extent of the temperature increase or decrease as well as to the area involved.

#### Microangiography

The same 24 rabbits as used in section B, were also subjected to microangiography. The ears were gently shaved and 0.1 ml of the solution to be examined was injected into the subperichondrial tissue with a 16-gauge needle. Each medium was cross deposited proximally in one ear, distally in the other ear.

The state of the ears was clinically evaluated and photographically recorded in transillumination for 7 subsequent days. The ears were also used for thermographic analysis during the same period.

One week after deposition the rabbits were anaesthetized with approx. 100 mg of Nembutal®

intravenously and microangiography performed. The thighs were shaved and the rabbit was positioned on its back. An incision was made through the inner surface of the thigh to expose the femoral artery. This was done with a fine polyethylene catheter (Appl. 2 ml of a 1:1 mixture of heparin (12.5% and 1/2% Xylocaine was introduced into the catheter. A 25% suspension of contrast agent Micropaque® (1 part barium to 4 parts physiological saline filtered through a Pyrex filter of porosity 1) was then introduced into the catheter at a controlled constant flow, of about 130 cm of water, which was kept at the arterial pressure of the rabbit. Both jugular veins were divided and the rabbit allowed to slowly exsanguinate over a period of 30 to 40 minutes as the Micropaque filled the intravascular space. During this time 4 more ml of the heparin-xylocaine mixture was introduced in divided doses. The Micropaque was continued about 30 minutes after the death of the rabbit. An average of 1 ml of Micropaque was introduced and 1 ml of blood and Micropaque were collected from the cut jugular veins. The ears were clamped at their bases with large compression forceps and severed from the head with the forceps.

The ears were placed outer side to Kodak MR X-ray plates which were covered with a thin aluminium foil. They were exposed to X-rays from a Machlett C X-ray tube at a distance of 120 mm set at 15 mA and 15 mg for 15 minutes.

## RESULTS

#### Vital microscopy, hamster cheek pouch

In the test situation with intact epithelium only drugs which in the other test situation showed severe damage caused severe microcirculatory disturbances such as stasis and microemboli.

In the other two experimental situations the microvascular disturbances caused by different drugs could be differentiated as



Fig. 2 (a, b) Hamster cheek pouch vital photomicrograph of venular system (a) before, and (b) after application of a drug belonging to group I causing slight micro-

vascular injury with some venular dilatation in (b) and increased hematocrit due to temporary slow-down of flow.

groups on basis of the severity of the damage to the microcirculation. In the first group, fairly moderate microvascular interference was a slow down of the flow mainly in the immediately after application of the drug. Occasional occurrence of spherocytes and red blood cells in the venular lumen, caused by hypo-osmolarity. Some white cells rolling alongside the venule walls and temporary standstill occurred in a few vessels. Changes were of a transient character and vascular function could be restored. A dilatation in the microvascular compartment occurred (Fig 3a, b).

In the second group the microvascular disturbance was more pronounced, with slow-down of flow in most of the microvessels and pronounced osmolar disturbances. However, when the drug was removed by irrigation with saline, flow was regained in most of the vessels. There was a distinct phase of hyperemia. White cells

were rolling alongside the endothelium, microthrombi and microemboli were characteristic features. Thus, the microvascular function was not completely restored and there were definite signs of microvascular injury (Fig 4a, b, c, d).

In the third group a severe injury to the microcirculation could be registered with immediate and complete blockage of flow and intravascular hemolysis. There was no restitution of microvascular function in this group (Fig 5a, b).

A clear dose-response situation is exemplified by tetracain which in 1% solution showed changes placing it into group II, but in 2% solution, to group III.

#### Macrophotographic registrations

Obvious classical inflammatory reaction around the deposit area could be registered for the substances causing severe tissue damage. In a few cases the central part of the deposit area was

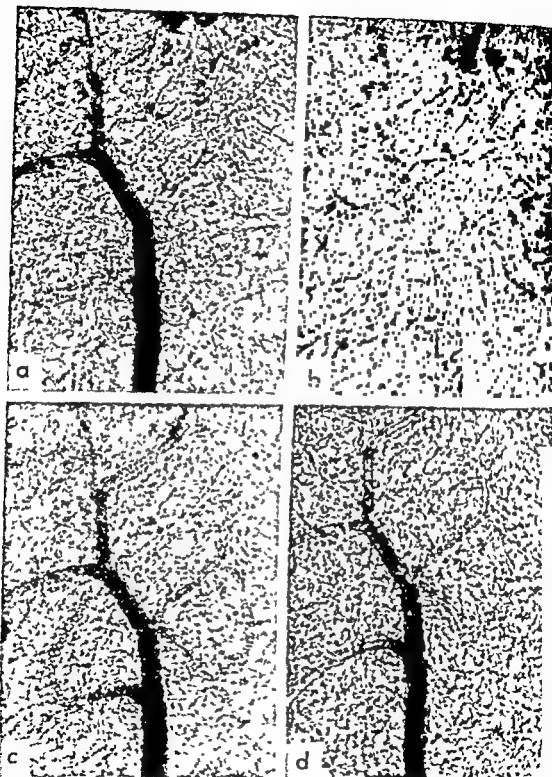


Fig 4 (a, b, c, d) Hamster cheek pouch Vital photo microgram of venular system (a) before, and (b) after irrigation with spherocytes in postcapillary venules. (c) Early phase with dilated microvessels and high blood flow. (d) Restitution phase after irrigation with spherocytes in postcapillary venules.



Fig. 6. a) b) Hamster cheek pouch. Vital photomicrograph of venular system (a) before and (b) after application of a drug belonging to group III causing complete

blockage of the microcirculation and permanent tissue damage. There was no restitution of flow after irrigation with saline.

at the end of the investigation. The presence of oedema and hemolysis often made a more detailed judgement of the underlying tissue condition impossible.

#### Microangiography

Microangiographic evaluation of microvascular reaction to subperiosteally deposited drugs was performed after 7 days. The lesions were characterized either by slight

microvascular proliferation, marked microvascular proliferation, or tissue loss and bordering vessel proliferation, respectively. These changes could be used to separate the drugs into three main groups, the first of which thus shows only minor microvascular damage, the second of which initiates definite damage of a proliferative character and the third of which causes loss of tissue and heavy marginal vessel proliferation (Fig. 6a, b, c).

#### 1. Microvascular injury caused by topically applied ENT drugs

	Group II Definite but reversible	Group III Severe non reversible
tin	Nafazolin Tetracain 1%.	Carbocain Thesat 4 1% Carbocain Thesat spray 5 2% Chloromycetin Topical Kenacort Mercuronorm Pyocetanin Tetracain 2% Xylocain spray 10%.
in Portr 1 Polymyxin B in 4		

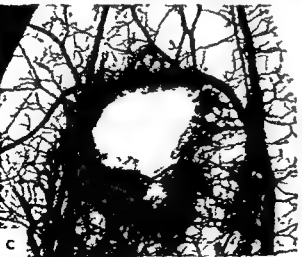


Fig 6 (a-c) Microangiograms of rabbit ear demonstrating in (a) slight microvascular proliferation caused by a drug belonging to group I in (b) some marginal vascular proliferation around central area of damaged tissue caused by a drug belonging to group II (c) Tissue necrosis surrounded by proliferating microvessels after application of a drug belonging to group III

### Infrared thermography

A stepwise reaction to the deposit could be registered. For almost all an initial decrease in heat emission, a deposit area could be seen. This turned to normal within one day for all substances without any sign of marginal emission indicating inflammation.

In another group the central temperature returned to normal within 3-4 days, parallel marginal increase of temperature, the deposit area and an increase of emission from areas outside the deposit. For some of the substances the central area did not return to normal during the registration period. The marginal temperature increase was vigorous and the emission from other parts of the ear strongly increased (Fig 7a-f).

According to changes in heat emission, drugs could be separated into three groups which were identical with the grading system used in vital microscopic studies.

Thus the information gained from experimental approaches were entirely new and could be summarized according to

### COMMENTS

This study of local tissue effects of applied ENT drugs has been performed in hamster's cheek pouch and rabbit's ear. Parameters have been considered relevant to immediate effects on the flow of blood in microvascular compartments. The consequences of the microvascular disturbance have also been evaluated. On the basis of the present investigation and on our previous studies of other drugs using a similar experimental set up, it appears justified to conclude that the point of view of the integrity of the ear of its restorative capacity and with respect to both normal and diseased tissue should be used with great caution. This comment is made with full appreciation of the fact that

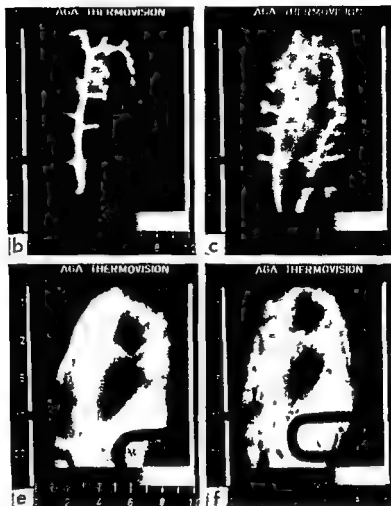


Fig. 1. Infrared thermograms of rabbit ear visualizing surface temperature. (a) and (d) represent the ear before deposition of drugs belonging to group I respectively. (b) and (e) show emission patterns before (c) and (f) after 7 days. Note minor changes

in sequence a-c in contrast to strong and irreversible changes in sequence d-f with marked central decrease of heat emission surrounded by patchy and vigorous increase of heat emission indicating inflammatory reaction.

from animal experiments cannot be readily transferred to clinical practice and

Drugs belonging to group II should be used with caution especially in cases where the drug is applied onto a tissue which is already injured. Drugs belonging to group I cause fairly little tissue damage, if there is no previous injury to the tissue. If however there are already vascular disturbances in a tissue even this additional injury can be an important factor influencing the healing capacity of the

Summing up then, this experimental analysis of topically applied ENT drugs has shown that some of the drugs used in clinical routine cause tissue necrosis, while others show distinct but reversible microvascular damage, whereas some drugs cause fairly little microcirculatory disturbance.

The methods used have proved to be sensitive enough to register even discrete tissue impairment at the microscopic level. It can be recommended that surface applied drugs as well as the vehicles involved be tested in this respect.



## ZUSAMMENFASSUNG

Die Gewebeschäden einer Gruppe ausgewählter, lokal applizierter Arzneien, die in der HNO Praxis verwendet werden, wurden analysiert. Die Analyse wurde mit Hilfe eines Testsystems, bestehend aus Vitalmikroskopie, Infrarot Thermographie und Mikroangiographie, durchgeführt. Durch Applizierung der Medikamente an Hamster und Kaninchen wurde eine Bewertung der Gewebeschäden erreicht. Dem Grad der Gewebeschäden entsprechend sind die getesteten Substanzen in drei Gruppen eingeteilt worden. Arzneien der Gruppe I verursachen nur sehr kleine mikrozirkulatorische Störungen, demgegenüber verursachen die der Gruppe II Gewebenekrosen. Das Endresultat unserer Untersuchung zeigt, dass lokalapplizierte Arzneien stets mit größter Vorsicht angewandt werden müssen, besonders wenn es sich um beschädigtes Gewebe handelt.

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## ULTRASTRUCTURAL FEATURES OF HUMAN JUVENILE LARYNGEAL PAPILLOMAS

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The ultrastructural organization of 12 juvenile laryngeal papillomas was investigated. Four patients were less than 40 years old but had carried the disease since childhood. The other 8 patients were 2-10 years old and had carried the disease from 1-5 years. There is regularly a discontinuity of the basement membrane and basal cells often exhibit abundant cytoplasmic protein. In the stratum spinosum there is often found inclusion bodies as well as Odland bodies and glycogen granules. In the superficial layers there are remnants as in parakeratosis. Virus like particles around 330 Å have been demonstrated in a stratified superficial cell. The scarcity of such particles is discussed with possible regard to a transformation taking place in the superficial layers of the papilloma and the high shedding rate of the surface cells.

Human laryngeal papilloma of the juvenile type is a benign epithelial tumour at the anterior part of the vocal cords and which has a tendency to recur along the epithelium, often involving the ary-region as well as the ventricular

part. Juvenile papillomas tend to recur after excision in some cases with intervals of one or two months. Permanent cure is often not achieved until adolescence and even then, persistent hoarseness of varying degree is not uncommon. There is reason to believe that the aetiology is viral but there is so far no convincing scientific evidence.

Papillomas of the adult type are far less apt to recur after excision. The aetiology is considered to differ from that of the juvenile type. A possible viral aetiology is not supported by scientific results, but cannot be excluded.

The purpose of the present study is to analyse the ultrastructural features of juvenile laryngeal papillomas and to discuss the possible pathological significance.

### MATERIAL AND METHODS

The material consists of biopsies from one normal and twelve pathological vocal cords of the human larynx. The specimens were excised under general anaesthesia at the Department of Otolaryngology, Karolinska Sjukhuset. The pathological material includes eight papillomas from children, and four papillomas from adults (Table 1). The latter group, however, can also be regarded as belonging to the juvenile type as they have persisted since childhood (Table 1).

Excised tissue was divided in two parts, one for histopathological diagnosis and one for electron microscopy. Specimens for light microscopy were fixed in a 10% solution of formaldehyde. Specimens for electron microscopy were fixed in a buffered 1% solution of osmium tetroxide according to Rhodin (1954). Specimens for electron microscopy were dehydrated in ethanol and embedded in Epon. They were sectioned with an Ultratome (LKB). Thin sections were collected on Formvar coated copper grids and stained with 1.5% uranyl acetate solution (Watson, 1958) and with lead citrate (Reynolds, 1963). Observations were made in a Siemens Elmiskop I operated at 80 kV.

Table I Juvenile laryngeal papillomas investigated

Age III biopsy (years)	Age at onset of disease	Sex		Total number
		Male	Female	
2-10	2-5	5	3	8
>40	<8	2	2	4

## OBSERVATIONS

*Normal material*

The basement membrane region The sub-epithelial connective tissue is loose in structure with vessels, cellular elements and scattered collagen fibrils (Fig 1) The connective tissue is separated from the epithelium by a continuous basement membrane about 600 Å thick (Fig 1)

Stratum basale The basal surface of the cuboidal basal cells is comparatively smooth due to the absence of cytoplasmic processes which appear in great number on cellular surfaces facing adjacent basal or spinous cells The cytoplasm (Fig 1) is structurally dominated by the high concentration of dense bundles of tonofilaments, some of which seem to be closely related to desmosomes and half-desmosomes In the cytoplasm there are also numerous round or ovoid mitochondria of high electron density, ribosomes interspaced between the tonofilament bundles, and a few vacuoles of varying dimensions In the supra nuclear region, numerous

all vesicles may be seen forming the Golgi system The nucleus of the basal cells (Fig 1) is large, forming an irregular sphere with wide as well as narrow indentations, furrows and clefts The nucleoli are well developed and the main part of the dense chromatin appears at the nuclear margin Intercellular spaces are narrow with numerous slender interdigitating cytoplasmic processes (Fig 2) The intercellular space is bridged by wide, tonofilament-containing processes from opposing cells joined by desmosomes In the basal cell layer the desmosomes are sparse White blood cells appear in the intercellular space They are not attached to the epithelial cells by desmosomes (Figs 1 and 2).

Stratum spinosum In this layer the general

structural features as described above are maintained However, the number of cytoplasmic processes is increased, compared with the basal layer Processes of the slender as well as wide types are numerous and there are desmosomes present

In the superficial part of the stratum spinosum the cells and their nuclei gradually assume a flattened shape Numerous Odland bodies (Odland, 1960) are seen close to the plasma membrane (Fig 3) Keratohyalin granules are absent Vacuoles with a moderately dense content appear in the cytoplasm (Fig 4).

Stratum superficiale In these superficial cells the nuclear chromatin is increased in density and the nuclear membrane is indistinct In the surface cells the nuclear remnants and the cytoplasmic structures are identified

*Pathologic material*

General features In all specimens the epithelium is of the stratified squamous type In the laryngeal papillomas the stratification is far less pronounced than in the normal tissue as the size

Fig 1 Normal larynx The basal cells (B) are separated from the connective tissue (Ct) by a continuous basement membrane (B/M) II BC White blood cell \* In the narrow intercellular space between basal cells there are numerous slender processes (arrow) Desmosomes (D) are few II BC White blood cell B/M Basement membrane × 9 000

Fig 2 Normal larynx Superficial part of stratum spinosum There are numerous Odland bodies (arrow)

Fig 3 Normal larynx Stratum spinosum (S) and stratum superficiale upper part of picture I II nuclear remnants × 9 000

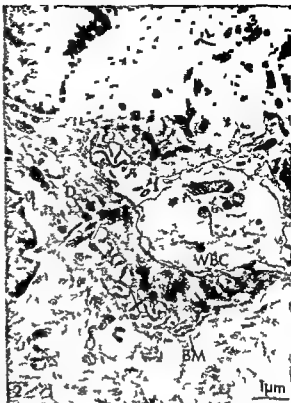
Fig 4 Laryngeal papilloma Basement membrane (B/M) Cytoplasmic material (C) is spread into the intercellular space

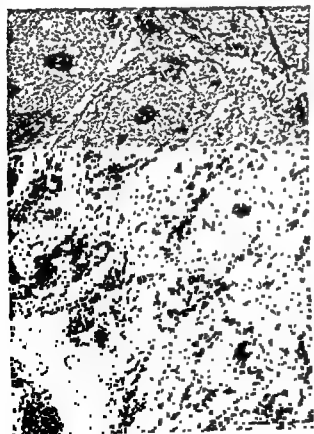
Fig 5 Laryngeal papilloma Stratum spinosum (S) and stratum superficiale upper part of picture I II nuclear remnants × 9 000

Fig 6 Laryngeal papilloma Stratum spinosum (S) and stratum superficiale upper part of picture I II nuclear remnants × 9 000

Fig 7 Laryngeal papilloma stratum basale. The basal cells are separated from the connective tissue by a continuous basement membrane

Fig 8 Laryngeal papilloma stratum spinosum. The cytoplasm of the basal cells is almost completely replaced by mitochondria (M) Ct Connective tissue × 5 000







(a) Laryngeal papilloma, lower spinosum. Heavy appearance of the glycogen like material (G) at lower  
 its of glycogen like material (G)  $\times 53\,000$  (b) Ap-  
 pearance of the glycogen like material (G) at lower  
 magnification  $\times 5\,300$



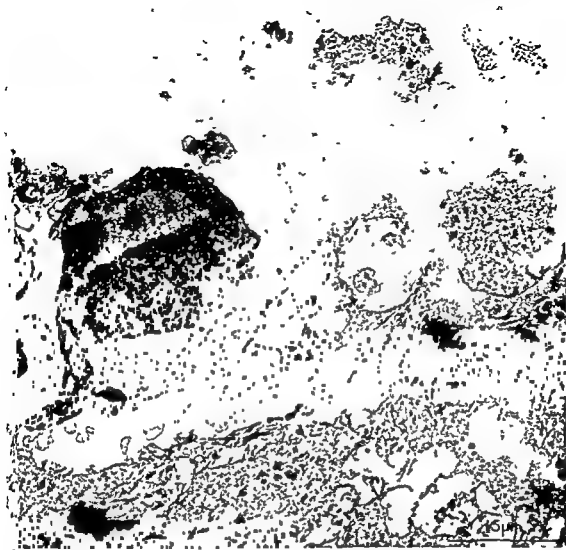
Fig 10 Laryngeal papilloma, stratum spinosum. A con-  
arrangement of cells is occasionally seen.  $\times 3\,000$

shape as well as the orientation of the cells vary. White blood cells present in the epithelium contribute to the irregularity of the pattern. The size of the cells seems to vary more than in the normal tissue. Cells smaller than normal are far more numerous than enlarged cells. The variability of the cytoplasmic and nuclear structure is markedly increased, compared with the normal tissue.

**The basement membrane region** The structural composition of the basement membrane is normal. It is frequently discontinuous,

however, and cytoplasmic processes basal cell extend into the connective tissue. In one specimen, cytoplasmic material is present in the connective tissue in an area where the basement membrane is absent and the plasma membrane of the basal cell is broken (Fig 11). The basal lamina is frequently duplicated in the irregular basal surface of the basal cell. Numerous white blood cells in places in the subepithelial area close to the basal cell.

**Stratum basale** The basal cells display different structural features in different spec-



1 Laryngeal papilloma. Atypical chromatin pattern in stratum spinosum.  $\times 6000$

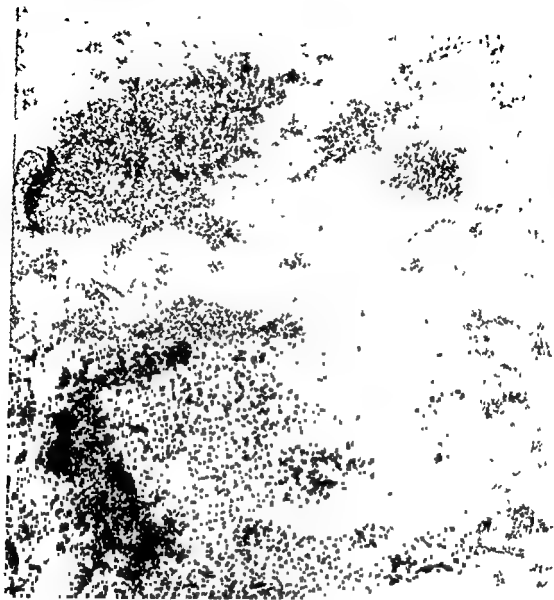
different areas. Some of them are highly electron-dense with a high concentration of tonofilaments and ribosomes (Fig. 6) and exhibit abundant cytoplasmic processes. The intercellular space is markedly increased in width in most specimens. Long and slender cytoplasmic processes extend from the basal cell. Wide-based, mitochondria-associated processes are rare (Fig. 7). In other basal cells the cytoplasm appears to be almost completely filled with mitochondria (Fig. 8). In two specimens heavy deposits of glycogen-like material appeared in the basal

cells (Fig. 9). The nuclei are ovoid in shape and, compared with the normal specimen, most of them appear swollen, with only traces of chromatin attached to the smooth nuclear margin (Figs. 7 and 8).

**Stratum spinosum.** Usually, the general types of structural patterns identified in the basal cell layer are maintained in the lower part of the stratum spinosum.

Abundance of mitochondria and glycogen-like material, wide intercellular spaces and the changed nuclear structure are features which,





when present in the basal layer, also appear in the lower spinous strata. In the central and superficial parts of the stratum spinosum some changes appear. In some specimens, spinous cells are seen concentrically arranged around a dense cluster of a few cells, thus distorting the regularity of the stratification (Fig. 10).

There are also cells with considerable deposits of chromatin arranged along the nuclear border and with atypically deposited chromatin (Fig. 11). In the superficial part of the stratum spin-

sum, Odland bodies appear and the cells usually tend to assume a flattened shape.

In one specimen several cells were found with a filamentous nuclear material leaking into the cytoplasm (Fig. 12).

**Stratum superficiale.** The flattened cells of the stratum superficiale are usually closely packed but in some areas the wide intercellular space is maintained throughout the epidermis.

The cytoplasmic organelles are identical in the surface cells. There are cytoplasmic



Laryngeal papilloma. In the dense cytoplasm of superficial cells, the organelles are still present. There is no keratinization.  $\times 5000$ .

vesicles and nuclear changes with loss of nuclear structures, increased density of the chromatin and disrupted nuclear membranes (Fig. 14). In one previously mentioned specimen (Fig. 14) "leakage" of nuclear material into the cytoplasm was seen also in several superficial cells (Fig. 14). These cells did not, however, display the typical changes usually seen associated with viral infection. Occasional round keratohyalin granules are seen (Fig. 15). In several disintegrated superficial cells there are round dense granules varying in diameter from 200–500 Å.

In one disintegrated superficial cell of a papilloma, numerous particles about 330 Å in size were seen (Fig. 15). Nuclear remnants are recognized even in the surface cells which are parakeratotic or nonkeratinized. The size distribution of the particles is seen in Fig. 16.

## DISCUSSION

### *Normal material*

The general ultrastructural features of the normal material are in agreement with the structure of the normal parakeratotic epithelium which



Fig 14 Same specimen as in Fig 12 Stratum superficiale Filamentous (f) nuclear material extends into the cytoplasm Nm Nuclear membrane  $\times 25\,000$

may be seen in certain parts of the oral cavity (Frithiof, 1969). The continuous and regular basement membrane, the abundance of tonofilaments and ribosomes in the basal cells contribute to this pattern. In the higher strata, numerous Odland bodies (Odland, 1960) are found. An obvious scarcity of keratohyalin material is a further characteristic. The presence of nuclear remnants and cytoplasmic organelles in the condensed cytoplasm of the surface cells is further evidence of the parakeratotic nature of the epithelium.

#### *Pathologic material*

In the laryngeal papilloma the epithelium is keratotic. There are structural changes in the basement membrane region. Similar changes are known to occur in a number of pathologic conditions involving epithelial tissues (T. Eriksson, 1972). The structural variations observed in all cell layers, is indicative of a pathological process. The abundance of mitochondria and heavy deposits of glycogen are evidence of metabolic disturbances. The nuclear changes, including atypically deposited chromatin,



415 Laryngeal papilloma. Part of a disintegrated nucleus (N) with virus-like particles (V). Kh, Keratin body. 5000. Integrated virus typical of a papilloma virus infected cell.

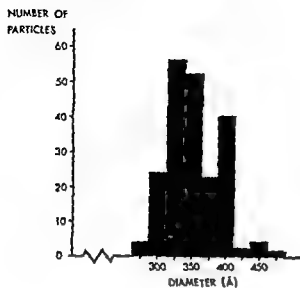


Fig 16 Size distribution of 200 virus like particles

age of filamentous nuclear material into the cytoplasm might be of interest when the possible viral etiology of the disease is discussed

It is generally accepted that papilloma (wart) viruses, belonging to the papovavirus group (Melnick, 1962) are the etiological agents in animal papillomas and human warts (De Monbreun & Goodpasture 1932, Shope, 1933). The ultrastructure of viral particles in human warts has been demonstrated by several authors (Almeida et al, 1962, Bunting 1953, Strauss et al, 1949, Williams et al, 1961). Similar virus like particles are found in superficial cells of human oral papillomas (Frithiof & Wersäll, 1967). It has been observed that the papilloma virus contain-

ing cells have a specific structure (Charles, Cheville & Olson, 1964)

In spite of a thorough search for virus particles in all 13 specimens only one superficial cell in one specimen revealed a pattern characteristic of virus infected or an aggregate of virus-like particles 330 Å in diameter. The size is in agreement with virus particles found in thin sections of warts and papillomas from human and rabbit skin and oral cavity (Table II). The shape and structure of the particles closely resemble the structure of loma virus in sectioned tissue. As no confirmatory virological experiments have been performed, the particles described should be referred to as "papilloma virus" on purely morphological grounds.

Previous reports on human laryngeal papillomas have not revealed any papilloma virus particles. Attention has been focused on plasmic particles with a diameter about 10 times that of the papilloma viruses (Mie & Schulz 1957, Timmel, 1961).

It is possible to consider the papilloma virus as an etiological agent of laryngeal papillomas in spite of the fact that virus like particles were found in only one cell in one specimen. Most of the viruses exert their influence on the germinative layer (Cheville & Olson 1962, Shope, 1962, Stone et al, 1959). It has been repeatedly stated in the literature that virus like particles are not seen in the basal cells (Frithiof 1962) deduced that animal tumour viruses including papilloma virus, are not always like, and that investigations should be extended to include a search for nonvirus like agents.

From the present study it is obvious that it is extremely difficult to find virus like particles in laryngeal papillomas. The reason may be the transformation of the unrecognizable virus particles presumably present in the basal and spinous cells, to the recognizable virus like particles. This occurs only rarely in laryngeal papillomas compared to a high shedding rate compared with the common wart in which virus like particles are easily demonstrated.

Table II Diameter of papilloma virus like particles in sectioned tissue

Author	Year	Particle diameter (Å)	Host and site of lesion
Bunting	1953	290	Human skin
Stone et al	1959	330	Rabbit skin
Charles	1960	330	Human skin
Williams et al	1961	340	Human skin
Rdzok et al	1966	380	Rabbit, oral cavity
Frithiof & Wersäll	1967	350	Human oral cavity
Present observation		330	Human larynx

suggested that the transformation of the into a recognizable shape occurs as a of a decreased or changed metabolism of superficial cells. Due to the high rate of production and shedding of the surface cells, the chances of viral transformation will render the force of recognizable virus-like particles a in juvenile laryngeal papillomas

## ZUSAMMENFASSUNG

Die ultrastrukturelle Organisation von 12 juvenilen Papillomen wurde untersucht. Vier Patienten waren 2-10 Jahre alt und hatten seit ihrer Kindheit an der Kehle gelitten. Die übrigen acht Patienten waren um 2-10 Jahren und hatten die Krankheit seit 1-5 Jahren. Regelmäßig wurde eine Diskontinuität der Basalmembran beobachtet, und die Basalzellen entwickelten zahlreiche zytoplasmatische Prozesse. Im Stratum corneum wurden oft glykogenartige Einschlüsse, Odontozyten und Keratohyalin-Granula gefunden. In den oberflächlichen Schichten fanden sich Kernreste wie bei wasserlöslichen Virusähnliche Partikel mit einem Durchmesser von ca. 330 Å wurden in einer desintegrierten Zelle festgestellt. Das seltene Vorkommen einer Partikel wird diskutiert, und dabei wird auf eine Transformation in den oberflächlichen Schichten des Papilloms und auf die hohe Abschleifungsrate der Epithelzellen hingewiesen.

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## EXPERIMENTAL STUDIES ON THE TREATMENT OF UNILATERAL VOCAL CORD PARALYSIS

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surrounded by connective tissue whereas the Silicon is enclosed in an unic membrane. We could not see a migration of the materials in the depth.

The injection of Teflon behind a paralysed vocal cord is a well known prognostic and therapeutic method. The reaction of the surrounding tissue to the foreign body has previously been described by Arnold (1962), Reichhardt (1970), Goff (1973) and others.

Besides Teflon we have made an experimental study of another material—Silicon. Both materials were tested in animals living under normal conditions. We wished to check whether parts

of the larynx, trachea or glottis are affected. Teflon was extremely favorable for this study, as its particles are birefringent and therefore easy to localise under polarised light. In three 12 week old lambs we injected Teflon paste behind one and Silicon liquid behind the other vocal cord. Silicon was sterilised immediately before the operation at a temperature of 120°C for 10 minutes. Temperature and length of sterilisation determine the viscosity of the Silicon liquid.

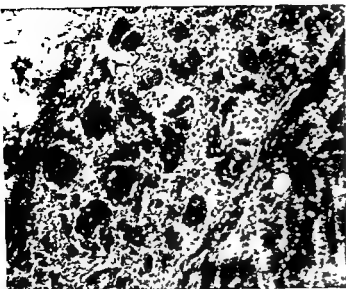
All the animals endured the operation well; they did not seem to have any trouble and developed normally afterwards. One of the sheep gave birth young after a normal pregnancy.

After 3, 6 and 10 months the animals were

killed, the larynx was removed and frozen in liquid nitrogen. Frozen  $30\text{ }\mu\text{m}$  were made and stained with eosin. Whereas the sectioning of injected side raised no problems, that of the Silicon injected side was difficult. Silicon was expelled during the procedure.

We found two types of tissue reaction to the Teflon paste: (1) The Teflon lay as a deposit encapsulated by a dense fibrous capsule and completely separated from the surrounding tissue (Fig. 1). Outside the capsule particles were visible (Fig. 2). This situation definitely explains the observation in the cheek of a hamster, inasmuch as the localisation of the Teflon deposit changed. Once walked in by the fistula, the foreign body can no longer migrate. (2) The foreign body could lie as a deposit neither enclosed by a dense capsule nor completely separated from the surrounding tissue (Fig. 3) as demonstrated by the muscle fibres passing through the Teflon particles (Fig. 4).

These two forms were not related to the time that the Teflon remained in the larynx; we found the first form in the animal at 3 and at 10 months, the second in the animal killed at 6 months. It seems far more important the site of injection, or the surrounding tissue, than the time of stay. Thus, we found the first form when Teflon was injected in fatty tissue, the second when



*Fig 1* Teflon deposit with strong connective capsule



*Fig 2* No Teflon particles visible outside the capsule under polarised light.



*Fig 3* Teflon deposit without capsule in between muscle tissue.



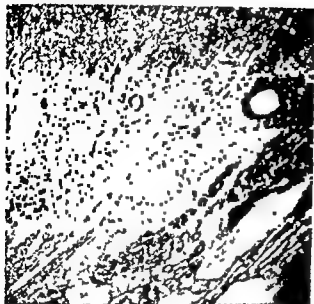


Fig 4 Muscle fibres (arrow) in Teflon deposit

tissue such as muscles or glands were present. Whereas the Teflon pushed aside the tissue in the first case, it infiltrated existing cleavage spaces in the second case.

The cytological reaction remained the same in both previously described types. The Teflon particles induced a strong conjunctive reaction, inasmuch as they were surrounded by the connective tissue (Fig 5). Furthermore, we noted a



Fig 6 Infiltration of histiocytes and lymphocytes

moderate infiltration of histiocytes and lymphocytes (Fig 6) and more or less giant foreign body type (Fig 7).

As concerns the migration of the particles, some of them were found at a distance from the large deposits (Fig 8). Studying their localisation in serial sections was obvious that they either remained in the canal of injection, or that they belonged to a more widely spread Teflon deposit, as

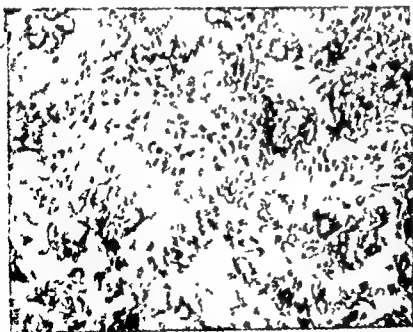


Fig 5 Connective reaction

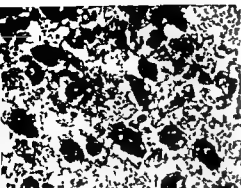
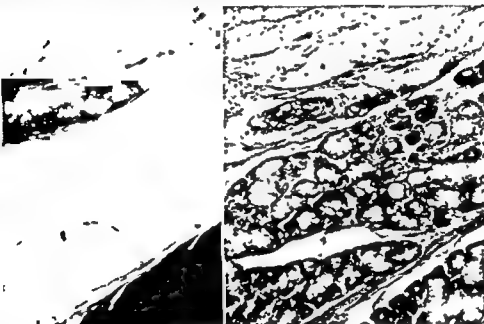


Fig 7 Giant cells of foreign body type



Particles of Teflon in the canal of injection

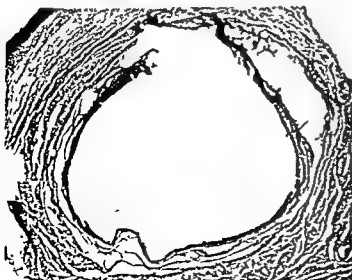


Fig 8 Pseudo-cyst caused by Silicon injection

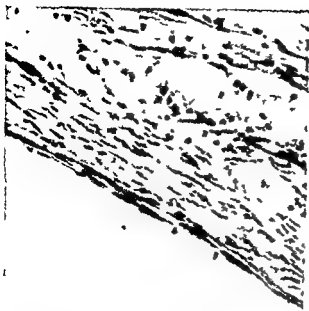


Fig. 10 Border of the Silicon pseudo-cyst

in form two. It was not possible to detect Teflon particles further away from the deposit nor in lymphatic spaces. It is therefore most probable that no migration of Teflon takes place.

As to the Silicon, we can only answer the question of reaction on the tissue. Beside the sections of the larynxes we also studied some sections of ears of rabbits. There was here a marked difference between Teflon and Silicon. The connective tissue did not infiltrate the Silicon nor did any cells migrate into the foreign body. The Silicon remained like a cyst in the tissue (Fig. 9). The wall of the cyst showed a certain increase of connective tissue but without forming a real capsule. A slight infiltration of histiocytes and lymphocytes was present. The border of the tissue looked like a pseudo endothelium with flat and long spread cells (Fig. 10).

We could not find any giant cells of the type

Silicon therefore seems to be better than Teflon, furthermore it may have the advantage of being removed more easily in expected reactions. Unfortunately it has not yet been set free for use on human beings.

## ZUSAMMENFASSUNG

Bei einem Patienten wurde hinter das eine Stimmband hinter das andere Silikonpaste injiziert. 10 Monaten wurden die Kehlköpfe histologisch untersucht. Dabei zeigte sich, dass die Teflonpseudokapseln bindegewebeartigen Kapseln eingewachsen waren, wenn sie im Fettgewebe lagen. War der Muskel oder Drüsengewebe erfolgt, blieb das primäre Gewebe zwischen den Kapseln noch erkennbar. Silikon löste nur eine leichte Zystenbildung aus und lag wie der Zysteninhalte in einer gewebeartigen Membran. Ein Abwandern des Silikon über Lymphspalten konnte nicht festgestellt werden.

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## HYPERPLASIA OF THE PAROTID GLAND UN S

### *A Stress Phenomenon?*

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The secretory function of the parotid glands as judged by the salivary secretion rate, has been studied in 10 patients with parotid hyperplasia UN S. Different levels of gustatory stimulation were used. The secretion rate was reduced in 8 out of 10 of the patients. At a moderately high level of stimulation but normal at maximum levels in all the 10 patients. The results suggest that the cause of the parotid hyperplasia may be increased sympathetic influence, possibly due to stress.

Enlargement of the salivary glands is a common feature of various gland disorders such as sialadenitis, tumours, obstruction to secretion, and sialosis. The etiology of sialosis is obscure and not well discussed, a typical feature is the involvement of the parotid glands, with bilateral swelling and an asymptomatic and often episodic course (Diamant, 1959, 1960, Rauch, 1959, 1962). Apart from the increase in gland size, radiological examination is usually of little help in the diagnosis, it may, however, exclude other clinical disorders with similar histological features. A characteristic of the histological picture is non-inflammatory swelling of the glandular cells (Seifert, 1964, Pohio, 1966). Parotid hyperplasia, has been observed in metabolic disorders, liver complaints, endocrine disorders, as a drug reaction, and nutritional deficiency (Rothbell & Duggan, 1957, Rauch, 1959, 1962, 1966, Borsanyi, 1962, 1963, Angerstein, 1963, Alappatt & Ananthachari, 1967, Ericson et al., 1969).

Parotid hyperplasia has been induced experimentally in dogs by adrenergic overstimulation with

repeated injection of isoprenaline and other catecholamines (Selye et al., 1961, Seifert, 1964, Pohio, 1966, Ohlin, 1966). The resulting cell picture bears a resemblance to that of spontaneous sialosis in man, Seifert (1964, 1966) has accordingly advanced the hypothesis that a possibly important etiological factor of sialosis in man is an increase in the sympathetic tone followed by disturbance of the normal enzyme synthesis in the salivary glands. It is not known, however, whether adrenergic stimulation has any causal connection with the sialosis observed in man. The purpose of the present pilot study was to examine the response of the parotid glands to gustatory stimulation of various strengths in cases of parotid hyperplasia UN S in order to find whether the condition is accompanied by disturbances in the vegetative influence of the glands.

### MATERIAL AND METHOD

The study was performed on 10 patients—5 men and 5 women—ranging in age from 26 to 56 years, that were seen at the Department of Otolaryngology, University Hospital, Umeå, for hyperplasia of the parotid gland. These patients constituted all the patients in whom a diagnosis of parotid gland hyperplasia UN S was made at the Department during the years 1965-72.

All the patients were submitted to a general medical examination, which, besides the routine examinations, included liver function tests, sero-

Table I *The pathological picture in 10 patients with parotid gland hyperplasia U N S*

- No symptoms  
 + Gland of moderate size moderate symptoms  
 ++ Large gland, marked symptoms

Case	Sex	Age	Durations of symptoms (years)	Enlargement of parotid		Xerostomia, subjective
				Right	Left	
1 KL	♂	41	1	++	++	-
2 AK	♂	56	3	+	+	-
3 GE	♂	41	1	+	+	+
4 RJ	♀	41	2	+	++	-
5 IH	♀	51	3	+	++	-
6 LC	♀	43	1	++	++	+
7 JE	♀	33	1	+	+	+
8 LE	♀	26	20	+	+	+
9 ES	♂	47	1	+	+	-
10 EJ	♀	41	4	+	+	-

logical analysis for the presence of collagenosis or sarcoidosis, tests for diabetes, a dietary history, and, in the case of the women, gynaecological examination. There was no conclusive evidence of any other disease or hormonal dysfunction and a diagnosis of parotid gland hyperplasia U N S was recorded. The duration of the disease and the symptoms are presented in Table I.

In addition to the clinical examination the parotid glands were radiographed and their secretory capacity was determined. The results were compared with those for a similar examination of 92 healthy control subjects; this material has been described elsewhere (Ericson, 1970, 1971). A sialometric examination of the parotid gland function was performed at relative rest and after oral stimulation with 1 and 6% citric acid. The gland function was judged to be normal or abnormal on the basis of a comparison with the values from the control group—both in absolute values and with account taken of the size of the glands.

The sialographic examination of the parotid gland included a determination of gland size and registration of changes in or near the excretory ducts. The gland size was represented by the area of the lateral projection of the gland on the sialogram. This area has been found to be closely correlated with the true volume of the gland ( $r=0.95$ , Ericson & Hedin, 1970). The

results of the sialographic examination also compared with those for the control

## RESULTS

The swelling of the parotid glands was with a duration of 3 to 4 weeks over a period of 6 months to 20 years. Besides the swelling and sensation of tenderness there was slight dryness of the mouth in some of the patients, recording a low secretion on stimulation with the 1% citric acid solution.

The pattern of the salivary ducts on the sialograms did not differ from that of the healthy glands, apart from the gland. There were no radiolucent zones or areas of dilatation. The branching of the ducts was normal. There were no deviations, destruction or alternations in the calibre of the ducts. No salivary calculus or strictures were seen. By the superficial position and size of the glands it was possible to see in all the patients a marked lateral extension of the gland on the anteroposterior sialograms, this corresponding to the clinically observed swelling.

The gland size, as represented by the lateral projection of the gland on the sialogram, is presented in Table II. All the glands (in one patient, case 8) were larger than the mean for the control group and in 10 patients the sizes were unilaterally and

II Area of projection of the parotid gland  
on sialograms in 10 patients with hyperplasia  
parotid gland U N S and the correlation  
the means for a control group of 92 healthy

	Area on lateral sialogram, cm <sup>2</sup>			Right-left difference
	Right	Left	Total	
group				
	22.6	24.6	47.2	2.0
	21.8	21.4	43.2	0.4
	22.0	21.0	43.0	1.0
	21.1	22.9	44.0	1.8
	26.3	19.6	45.9	6.7
	22.5	22.6	45.1	0.1
	18.8	16.7	35.5	2.1
	11.6	11.8	23.4	0.2
	24.9	23.8	48.7	1.1
	17.8	17.8	35.6	0.0
	20.9	20.2	41.0	1.5
	4.3	3.9	7.7	2.0
group				
	15.5	15.6	31.1	0.9
	2.7	2.9	5.4	0.9
ion analysis				
	3.25	2.88	3.15	0.78
ance	++	+	-	Not sign

the normal limits, 20.9 cm<sup>2</sup> (Ericson, 1970). The difference in mean gland size in the hyperplastic patients and the control group was statistically significant, as represented by the projected area on the sialograms.

In order to find whether the parotid glands on one side in a given patient were differently affected by the disease the left-right difference was calculated (Table II). In only one patient (5) was this difference greater than what was regarded as normal—namely a maximum of 7 cm<sup>2</sup> (Ericson, 1972), this indicates a predominantly similar development of the glands on both sides.

The secretion rate of the parotid gland on stimulation with 1 and 6% citric acid is illustrated in Fig. 1. There was a large individual variability; however, in comparison with the healthy controls, the secretion rate being on average lower for the 1% but higher for the 6%.

**citric acid** The differences between the means were not significant, however. For the 6% solution the level of significance for the difference was  $P < 0.10$ . The secretion at rest, which was also recorded, displayed no difference from the control values.

As the secretion rate is normally related to the gland size, the gland function for the 10 pairs was analysed with respect to this parameter. The results are shown graphically in Fig. 1, here the limits of the normal range—with a 95% probability based on the control group—have been entered. It is seen that on stimulation with the 1% citric acid solution, 8 of the 10 pairs displayed a lower secretion rate than normal for the gland size, whereas with the 6% solution the secretion rate was practically normal. The 2 patients (cases 1 and 9) recording a normal secretion rate for the lower strength had the largest glands.

## DISCUSSION

In 7 of the 10 patients the size of the parotid glands as represented by the lateral projected area on sialograms was greater than is normally seen in healthy persons (Ericson, 1973). Except for deviations in size the sialographic appearances of the gland did not differ from those of healthy glands. Solely on the basis of the sialograms it is therefore impossible to distinguish the glands in the patients with parotid hyperplasia from normal but extremely large glands. The remaining 3 patients with moderately large parotid glands did not show differences from the normal sialographic picture. The analysis of the relationship between the secretion rate and gland size as represented by the projected area on lateral sialograms, however, suggests that there were defects in the glands in 8 of the 10 patients. In 2 of them (cases 1 and 9) it was impossible to decide whether the glands were affected by pathological alterations or whether they were only large in size, this being suddenly noticed by the patient. Nor can an incorrect diagnosis be ruled out, since neither the clinical nor the radiological criteria for a

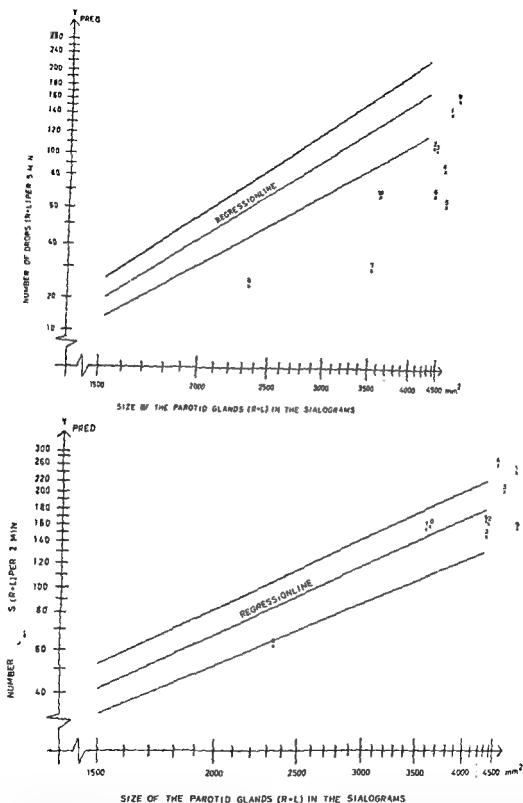


Fig 1 The rate of secretion of the parotid glands on stimulation with citric acid solution versus gland size, in 10 patients with asymptomatic gland enlargement. The upper and lower lines represent the normal range

for healthy glands (95% confidence interval). The case numbers on the curves are case numbers. Upper graph 1% citric acid solution. Lower graph 6% citric acid solution. 1 drop is equivalent to 0.06 g of saliva.

sis of parotid hyperplasia are definite to the margin of error inherent in a dry examination, the episodic nature of disease and the usually large inter-subjectivity in gland size.

1 of the 10 patients comprising this series showed a clear tendency for a low secretion rate to be associated with a low or submaximal stimulation level, but a normal secretion rate with submaximal stimulation. The difference in the secretion rate of the diseased and healthy glands of corresponding size for different stimulus levels is so large that it is feasible that there is an organic cause for the difference in the glands' reaction. It is, however, inconceivable that this factor is produced due to the demonstrable morphological changes in the gland parenchyma, since the reaction to the different stimuli would presumably be uniform.

It is more likely to be due to a disturbance in the nervous regulation of the gland function, as has been demonstrated experimentally in the rat, where an increased sympathetic tone inhibits parasympathetically elicited salivation and gland cells deprived of their parasympathetic control become hypersensitive to certain chemical substances such as adrenalin and isoproterenol (Richins & Kuntz, 1953; Emmelin, 1966). The function test would seem to indicate that parotid hyperplasia is due to increased sensitivity of gland cells resulting from increased sympathetic influence (possibly via a stress factor). This increased influence may at a low level of stimulation partially block the parasympathetic system but at maximum stimulation level be overcome. The support for this hypothesis is found in experiments in which sialosis was induced in serous glands in the rat (Selye et al., 1961, 1962, 1964; Wells & Peronace, 1964; Ohlin, 1966). Stress can elicit morphologically demonstrable reactions in salivary glands as has been shown by, among others, Seifert & Seifert (1948). Seifert and Pohjo have suggested the following mechanism underlying parotid hyperplasia in hyperstimulation, isoproterenol and certain other biogenic catecholamines

disturb the protein synthesis in the serous salivary glands and produce an enlargement of the acinar cells by increasing the endoplasmic reticulum and vacuole formation in the granules and Golgi apparatus.

The salivary glands are to a marked degree species specific and observations on one species are not always applicable to another. Even so, the possibility that catecholamines exert an influence on the serous salivary glands in man cannot be excluded, and there is evidence of similarities in the histological and histochemical features of sialosis induced in animals and spontaneous sialosis in man. A disorder of the vegetative nervous system—due to, for instance, stress—may therefore be one causal factor in certain forms of sialosis. It has been established that the salivary glands in man react to stress and modify the amount of cholinesterase-like substances and electrolytes in the saliva (Prader et al., 1955; Giddon & Lisanti, 1962). In the rat, stress causes a reduction in the number of granules in the salivary gland acini (Roseman et al., 1960).

A careful examination of the patient's social situation may be one approach to the cause of asymptomatic parotid hyperplasia and provide a guide to both diagnosis and treatment.

## ZUSAMMENFASSUNG

Bei zehn Patienten mit der Diagnose Parotishyperplasie U N S wurde die sekretorische Kapazität der Parotis speicheldrüsen genau untersucht. Verschiedene Grade von Geschmacksreizung wurden verwendet. Bei einer moderaten Reizung war die Speichelsekretion bei acht von zehn Patienten reduziert. Bei submaximaler Reizung war die Sekretion normal. Die Resultate werden diskutiert. Ein erhöhter Sympathikustonus wahrscheinlich auf Stress beruhend wird als Ursache von Parotishyperplasie U N S angegeben, aber es existieren auch andere Ursachen.

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## RECENT ADVANCES IN MICROSURGERY OF THE MAXILLARY ANTRUM

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A new and more detailed approach to the maxillary sinus is described. It has the advantage of avoiding the canine fossa and potential dental trauma. The procedure is performed intranasally and is associated by less morbidity than with the Caldwell-Luc operation. The opening into the sinus is small, thus avoiding bone removal. However, the interior of the sinus can be visualized sufficiently well to justify the procedure. A sinuscope and operating microscope compensate for the diminished exposure. In the majority of cases, antral surgery is carried out for chronic sinusitis, and this procedure conforms to basic principles of adequate drainage and exteriorization of the sinus contents into the nasal cavity.

The surgical treatment of chronic maxillary sinusitis by the Caldwell-Luc and the intranasal antrostomy have been standard operations for many years and are still widely used. However, these procedures have shown certain limitations and disadvantages.

The purpose of this paper is to present a new and more appropriate approach to the maxillary sinus employing the operating microscope and the sinuscope. The operating microscope, which has revolutionized otological surgery, and more recently improved the caliber of endolaryngeal surgery, has been neglected in surgery of the maxillary sinus. Only in the past few years has the microscope been applied to surgery of the antrum.

The Caldwell-Luc or radical antrum operation has as its main advantage a good operating field. The problem with this procedure is potential damage to the teeth and sometimes considerable morbidity. This operation requires an incision in the gingivo-labial sulcus almost 2 inches in length. To expose the anterior wall of the

maxilla and canine fossa entails considerable dissection and elevation of the soft tissues of the cheek, with exposure and retraction of the infra-orbital nerve. This often results in considerable postoperative swelling of the cheek and, not infrequently, numbness over the distribution of the nerve. The more important problem results from removal of bone in the canine fossa, which may result in damage to the teeth. Sometimes bone is taken down so close to the teeth that occasionally the apex of a tooth has been inadvertently violated. This approach does not interfere in the same degree with the nerve or blood supply of the teeth.

A brief review of the innervation of the upper teeth is appropriate (Fig 1). All of the upper teeth are supplied by branches of the maxillary nerve. One of the main nerve supplies consists of the infra-orbital nerve which comes down through the anterior wall of the maxilla.

Harrison reviewed the radical antrum surgery in a series of 96 patients, and found temporary paresthesia in over 70% of cases. There were 16 patients who had permanently insensitive teeth. The recovery of sensation that occurs in most teeth is explained by the fact that all three superior alveolar nerves contribute to the alveolar plexus. The blood supply of the upper teeth is often irreparably damaged when radical surgery is employed. We have also seen many patients who have complained bitterly post-operatively, and have known dentists who have reported missing apices on extracted teeth in patients who had undergone Caldwell-Luc surgery.



One of the objectives of the Caldwell-Luc operation has been the removal of the membranous lining of the antrum though experience has shown that once adequate drainage has been established, diseased sinus mucus membrane will usually heal, and need not be removed. In most cases the establishment of an antrostomy is all that is required.

The intranasal antrostomy operation first described by John Hunter and popularized by VanAlyea is difficult to perform well. It is a blind procedure which is accompanied by considerable bleeding and does not allow for inspection of the sinus contents. The antrostomy window often tends to close rapidly. Many prominent authors have recommended that the intranasal operation should be abandoned.

As a result of the drawbacks with these procedures a number of other approaches to the maxillary sinus have been suggested. Harrison recommends a middle meatal approach with abandonment of the intranasal antrostomy beneath the inferior turbinate, and only occasional

use of the Caldwell-Luc. The author thinks the middle meatal approach to chronic sinus disease does not provide dependable drainage and will interfere with the natural physiological functions of the sinus. Sanderson has described the use of a plastic flap from the anterior antral canine fossa through which he introduced a sinuscope. However, this is of no advantage since it also violates the blood supply to the teeth.

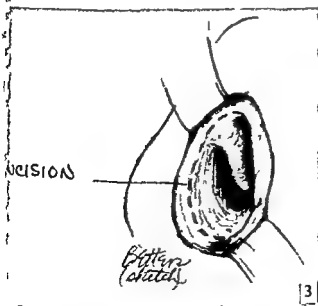
Our approach to the maxillary sinus is nasally by means of a vestibular incision through the ascending process of the maxilla. The canine fossa is not violated and a permanent antrostomy remains to provide drainage. Through the opening a sinuscope can be introduced for complete visualization and examination and the operating microscope can also be used to inspect the antrum and aid in the removal of the anteronasal wall from before back.



ions

indications for our new approach are as follows:

- chronic sinus disease
- radiographic examination
- biopsy of tumours
- removal of polyps



- 5 Removal of irreversibly diseased areas of mucus membrane
- Removal of tooth roots or other foreign bodies

### Procedure

The stages of the operation are as follows

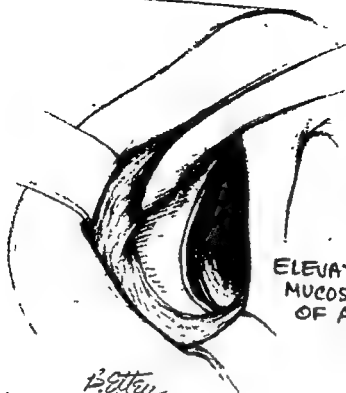
1 The innervation of the anteronasal wall is blocked with Xylocaine 1% and Epinephrine 1-30 000

2 The inferior turbinate is infractured (Fig 2)

3 A hockey stick incision is made along the anterior edge of the ascending process (Fig 3) of the maxilla from the anterior attachment of the inferior turbinate downwards to the nasal floor

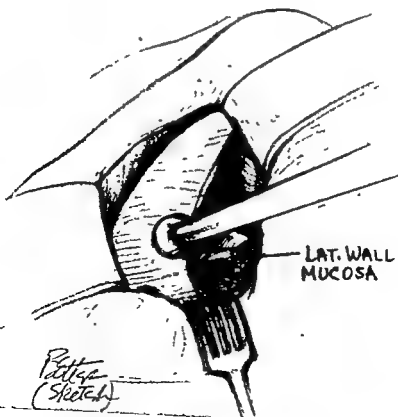
4 A lateral incision is then made to the periosteum from the vertical incision below the attachment of the inferior turbinate about 1.5 cm long, curving downwards to the nasal floor. Using a periosteal elevator, a flap is raised (Fig 4)

5 Using a drill, the maxillary antrum is entered anteromedially and the antero-nasal wall is taken down under direct vision, using a Zeiss microscope (Fig 5). The edges of the new opening are rounded off. The sinuscope is inserted and the sinus cavity carefully examined,



ELEVATION OF  
MUCOSA AND  
OF ANTERO-

*P. E. E. (Sketch)*

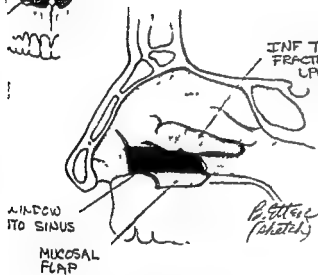


LAT. WALL  
MUCOSA

*P. E. E. (Sketch)*

6

7



8



irrigated and suctioned. Photography is carried out, and biopsies may be taken (Figs 6 and 7 demonstrating an antral lesion).

6 Using the microscope and dental drill, the anteronasal window is created from before back wards under direct vision (Fig 8).

7 A plastic Teflon prosthesis of variable size may be inserted to maintain the opening and facilitate further irrigation or manipulation without discomfort (Fig 9).

8 The flap is replaced, and the inferior turbinate is moved to the original position.

9 If a prosthesis is used it should be removed after about 2 months.

## RESULTS

We have carried out 70 operations of this kind and although the technique originally seemed difficult, most of the problems have been solved. Following the surgery there is some dental numbness but its duration is short lived.

## CONCLUSION

A new operation is described which is less traumatic than the Caldwell-Luc procedure and more effective than the intranasal antrostomy in surgical treatment of chronic maxillary sinus disease. The antrum is approached through an intranasal vestibular incision entering the lower part of the ascending process of the maxilla.

Through this opening the sinuscope etc. and the microscope is also used to create adequate anteronasal window.

Finally, I deem it appropriate to quote a quotation from a well known orthodontic book *Extensile Exposure* by A K Henry. Henry was one of my professors at medical school for whom I have great respect.

"'Tis not so deep as a well nor so  
a church door but 'twill serve."

## ZUSAMMENFASSUNG

Es wird eine neue Operation für chronische Höhlenerkrankungen beschrieben die weniger traumatisch ist als die Caldwell-Luc Operation und erfolgreicher als die intranasale Antrostomie. Wenn das Zupfen der Gewebe unzureichend ist ist eine radikaleren angebracht (L L F).

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## DAMAGE TO THE STRIA VASCULARIS IN THE GUINEA PIG BY ACUTE ATOXYL INTOXICATION

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The aim of the present study was to show the optical degeneration at the ultrastructural level during the stria vascularis experimentally. The changes after acute atoxyl intoxication occur after 1 hour and begin as a degeneration of both the I and the intermediate cells, whereas the basal part mainly unaffected. The severely damaged or intermediate cells may become loosened from the stria vascularis and rejected from it into the endolymphatic space. Under such conditions the basal cells of the surface facing the endolymph, although they rarely initially there may be a slight bulging of Reissner's membrane, but soon the membrane is depressed and sometimes a total collapse occurs. Reissner's membrane flattened over the tectorial membrane against the organ of Corti. It is only when Reissner's membrane touches the stria, secondary degeneration with formation of intracellular inclusion bodies is an interesting early stage of the damage pattern.

Alphonse Corti described the stria vascularis as a capillary network closely lined by epithelial cells. Nowadays it is known that three different types of cells are found in the stria: the dark (apical or marginal), the light (chromophil or intermediate) cells, and the basal cells (Fieandt & Saxen 1937; Engstrom 1955; Smith 1957; Bairati & Iurato 1957). On the basis of the ultrastructural features it may be assumed that the stria is an extremely highly differentiated tissue, is able to

perform a selective secretory activity and to a lesser extent, some resorptive function (Sjoendin 1967).

In 1938 Hallpike & Cairns were the first to describe the histological picture in Meniere's disease. They found gross dilatation of the scala media and degeneration of both Corti's organ and the stria vascularis.

Recently the functional pathology of the stria vascularis has aroused renewed attention following the demonstration of stria damage in experimental animals treated with ethacrynic acid by Quick & Duvall (1970). Nakai (1971) also reported stria changes due to ethacrynic acid.

The toxic effect of the arsenic compound atoxyl on inner ear structures was described by Miyamoto (1931). He found histopathologic abnormalities in the organ of Corti and the stria vascularis. Nakamura (1935), Ozeki (1937) and Ruedi (1951) confirmed these findings. The effects of arsacetin (acetylated atoxyl) were first studied by Causse et al (1940) who recorded loss of the Preyer's reflex and the cochlear microphonics and delayed vestibular disturbance. Similar findings were obtained by Leonard et al (1971).

Johansen (1953) reported marked histopathologic changes after arsacetin administration involving the organ of Corti and the stria vascularis.

The purpose of the present study was to

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Table 1 Diagram illustrating the doses injected into the guinea pigs of the experiment 2

No	Mg/kg at each injection	Number of injections	Injected during (days)	Totally mg/kg	Killed after the last injection (days)	Preyer reflex
20	70	3	2	210	1	-
52	70	2	1	140	1	-
53	100	1		100	1	(+)
54	70	2	1	140	1	(+)
55	140	1		140	1	-
63	140	1		140	1	-

investigate the toxic effects of atoxyl on the stria vascularis with special regards to the question whether atoxyl is a suitable substance for experimental studies of stria function and pathology

## MATERIALS AND METHODS

Nine young, healthy guinea pigs, with normal Preyer's reflex, weighing around 250–350 grams, were used for the experiment. Six animals were injected with atoxyl. The control group consisted of 3 healthy, non injected guinea pigs.

### Administration of atoxyl

Each animal was given a 2% solution of atoxyl in sterile water subcutaneously during a certain period of time. The dosage varied between 70 and 140 mg atoxyl per kg body weight. The total dose ranged from 100 to 210 mg per kg body weight. The duration of administration varied between 6 hours and 2 days and the survival time before decapitation between 6 hours and 1 day after the last injection (Table 1).

### Morphological procedures

For microscopy, specimens were taken according to the technique of Wersäll (1956) with

decapitation of the animal. The temporal bones were removed, and an opening made in the bulla tympanica. The stria was extracted, the round window re-opened, and a small opening made at it to allow sufficient penetration for fixative. The cochlea was perfused with 2% osmium tetroxide solution through the opening in the apical coil of the cochlea. The stria was immersed for 2 hours in the fixative, then dehydrated in alcohol, and embedded in paraffin.  $1\ \mu\text{m}$  thick sections stained with toluidine blue, were studied in a light microscope. Thin sections were stained with uranyl acetate and lead acetate.

## RESULTS

Structural alterations may be observed in some parts of the stria vascularis even 6 hours after the administration of atoxyl. After 24 hours severe degeneration occurs in all the turns of the cochlea.

After treatment with 70 mg atoxyl per kg body weight, and repeating the same dose after 6 hours the animal was killed 12 hours after the first injection. The apical turns were severely affected whereas the basal coil showed only minimal changes. The same changes



(a-d) Different stages of stria degeneration. Light microscop. Magnification 280 $\times$ . The stria vascularis im- become swollen (a: 3rd coil animal No. 20) and marginal and intermediate cells are rejected from the stria surface (b = 3rd coil animal No. 57). The position of Reissner's membrane is altered and it becomes depressed and lies over the organ of Corti in the final stage (d: 3rd coil animal No. 54).



Fig 2 Heavily degenerating intermediate (I) cells in the stria vascularis. The blood vessel (B) is blocked by red blood cells (3rd coil, animal No. 20). EMG

ved when 140 mg atoxyl per kg body t were administered, and the investiga- was made 24 hours after the injection vations after only 12 hours showed an t normal morphological structure. An in- h of 100 mg atoxyl per kg body weight, he specimen studied after 24 hours, d only minimal degeneration in the stria aris, except in the apical coil. Severe eration of the stria vascularis occurred 70 mg atoxyl per kg body weight was istered three times within 36 hours, and unea pig killed 48 hours after the first on. In these specimens the stria of all the as so severely damaged that no coil was ved to predominate. The damage areas e localized or diffused. Lesions occurred both the blood vessels in the stria vas- , and the spiral prominence, the latter ie most frequent site. Among the three of stria cells, the marginal and in- iate cells were most frequently affected

degeneration of marginal and in- iate cells followed the same pattern. nt degrees of vacuolization appears Figs 2 and 3 b) which may become so ve that almost the whole cell is filled up uoles, and the degenerating cell organel- nucleus are pressed towards the periph- arts of the cell body. The normal cell ance, with cytoplasm densely packed itochondria, especially in the basal part, olgi membranes, was lost at an early during degeneration. The normally ir- r, chromatin rich nucleus was the last nent to disintegrate by swelling, chro- fragmentation and, finally, by rupture of uclear membrane. The mitochondria go swelling, and accumulation of dense al occurred between the cristae (Fig. One or more such accumulations can be in almost every damaged mitochon- , but they occupy only a minor portion. These changes occur relatively soon in ital phase of mitochondrial degeneration the cristae mitochondria are still

often preserved, although they are irregular and fragmented. In some cases the dense material occupied a major part of the mitochondrial volume. In a few cases mito- chondrial degeneration, with formation of lamellated bodies, occurred.

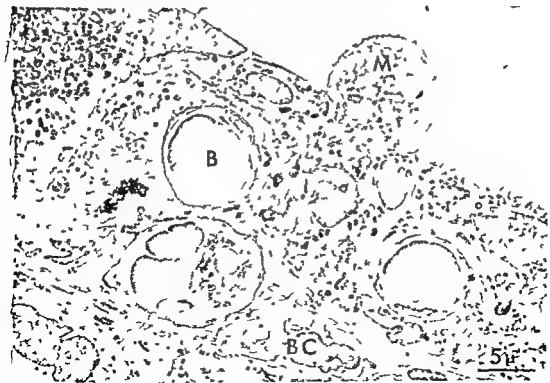
While these degenerative processes were taking place there was a tendency for the rejection of the severely damaged cells from the stria vascularis into the endolymph (Fig. 4 a, b). Not all the marginal cells, however, that become damaged undergo all the phases of degeneration before they are loosened from the stria. A cell may be rejected also at an earlier stage, whereas some cell organelles and fragments of the nucleus may still be recognized.

The lining facing the endolymph will, there- fore, consist of both marginal and intermediate cells and, to a lesser degree, also of basal cells. The loosening of the marginal cells from the stria vascularis is more manifest towards the spiral prominence, but is also observed around the vessels in the stria to a great extent. The area around the insertion of Reissner's membrane is not so severely affected. Some of the rejected cells are so severely damaged that it is impossible to recognize their origin (Fig. 5). There are findings which indicate that the intermediate cells may be affected first. In several serial sections starting at the stria vascularis and proceeding towards the spiral ganglion rather severely degenerating intermediate cells may be observed, whereas the processes of the marginal cells appear to be only slightly damaged.

In the cell cytoplasm of many intermediate cells there are accumulations of colloid like, dense material (Fig. 3 b) of the size of several mitochondria. Nothing of the ordinary endo- plasmatic reticulum or ribosomes can be observed. Severely damaged intermediate cells are rejected from the stria vascularis in the same way as in the marginal cells. The melanin pigment participates in the rejection process either as a component of the rejected cell or it may also be found in the endolymph



Fig 3 (a) Intermediate cell with vesiculation and degenerating mitochondria (M) containing electron-dense material (3rd coil animal No 20) EMG (b) Intermediate cell with lipid like material (L) in the cytoplasm, vesiculation and mitochondrial degeneration (3rd coil animal No 20) EMG



(a) Advanced stage of the rejection of heavily staining marginal and intermediate cells (2nd coil No 57) EMG (b) Early stage of the rejection of a moderately damaged marginal (M) cell. The blood vessels (B) are blocked by red blood cells. The basal cells (BC) appear normal (2nd coil an mal No 54) EMG



Fig 5 Loosening of heavily degenerating marginal and intermediate cells from the surface of the stria vasculans

The basal cells BC remain unaffected (3rd case No 57) EMG

7, probably as the result of a completely integrated cell

the flat, tightly packed basal cells, with sometimes gigantic intracellular vacuoles are very little affected by acute atoxyl intoxication. However, not until the marginal and the intermediate cells have degenerated become loosened from the stria vascularis, and the adjacent marginal and intermediate cells are unable to stretch and fill the missing cell spaces, do the basal cells become involved in the lining towards the endolymphatic space.

■ stria vessels are often blocked by accumulations of blood cells. In a few capillaries, small lamellated bodies may be observed near the nucleus in the endothelium. In acute atoxyl intoxication an intercellular space around the blood vessels in the stria vascularis is a rather constant finding (Fig. 6). Such space is found in the control group during the early stages of atoxyl administration. Reissner's membrane initially becomes slightly bulging, but this stage is soon followed by depression of the membrane, so that it becomes flattened over the tectorial membrane of the organ of Corti. When Reissner's membrane is collapsed it covers the tectorial membrane and the Hensen cells, but it very rarely touches the stria vascularis itself (Fig. 7). Although all the coils of the cochlea are examined, the findings are more pronounced at the apical coils. Between this extreme position of Reissner's membrane, as described above, and its normal position all kinds of intermediate stages may be found.

## DISCUSSION

The present investigation shows that the stria vascularis in atoxyl treated guinea pigs undergoes different stages of degeneration of the marginal and the intermediate cells. The stria initially appear normal under the light microscope but under the electron microscope morphological alternations are dis-

cernible. Among the stria cells the earliest and most frequently affected are both the marginal and the intermediate cells and it has so far not been possible to assert with certainty which cell type degenerates first. However, in some specimens the intermediate cells are more severely affected than the marginal cells.

Both marginal and intermediate cells may become loosened from the stria vascularis and float out into the endolymph. At the same time, pigment is also liberated, so that it flows out into the endolymph. Here and there, cells may be seen to burst and discharge their content of pigment. The degeneration of the stria vascularis may occur in all parts of the true stria vascularis, although there is a predilection for the zone near the spiral prominence, and that near the blood vessels. The reason for the vulnerability of these areas is not clear but it is likely that there may be a high concentration of the substance around the vessels.

Mygind & Dederling (1932) and Hallpike & Cairns (1938) were the first to describe the distention of the cochlear duct in histological specimens, as evidenced by the bulging of displaced Reissner's membrane. These findings have been confirmed by Hallpike & Wright (1940), Rollin (1940), Lipman (1942) and Altman & Fowler (1943), so that the term 'endolymphatic hydrops' is generally accepted as a synonym for Meniere's disease. In the acute atoxyl intoxicated guinea pig there is a tendency for a slight hydrops to occur initially but the slightly bulging Reissner's membrane is very soon depressed, and subsequently it collapses to a considerable extent. Altman (1961) stated that the dilatation of the endolymphatic system appears to be the result of an increased endolymphatic pressure probably due to a change in the chemical constitution of the endolymph by an accumulation of molecules causing a light osmotic pressure in the endolymph. In acute atoxyl intoxication the initial phase with hydrops is extremely short in relation to the phase of stria cell degeneration.

Quick & Duvall (1970) reported changes in



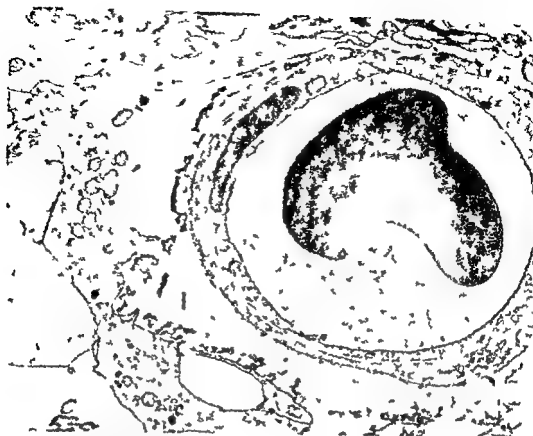


Fig. 6. Strial blood vessel with surrounding intercellular space (l) (endothelium) and (i) (intercellular space) EMG.

the stria vascularis after high doses of ethacrynic acid whereas smaller doses did not produce any anomalies. There was an increase in the thickness of the stria vascularis due to intracellular and extracellular edema. The marginal and the basal cells looked normal. The intermediate cells were either completely destroyed or in an advanced stage of atrophy. The melanin granules normally contained within the intermediate cells seemed resistant to atrophy.

Nakai (1971) reported, however, that ethacrynic acid intoxication mainly affected the marginal cells of the stria vascularis. Contrary to the damage to the sensory cells in the organ of Corti, the changes in the stria vascularis had disappeared in cases that were observed for long periods after final administration. Whereas the ethacrynic acid degeneration of the strial cells apparently is reversible

in the early stage of toxic reaction, the changes found after atoxyl intoxication are irreversible type.

Kimura & Schuknecht (1970) found in patients with Meniere's disease that the most frequent changes were in the marginal cells by decrease in cytoplasmic reduction in the number of mitochondria, pinocytotic vesicles and rough endoplasmic reticulum. The intermediate cells were frequently affected but similar changes were not observed. As the marginal cell layer thinned, the intermediate cells grew and occupied a major portion of the stria. The basal cells were least affected. When the marginal and intermediate cells disappeared, the basal cells often remained intact, thus the endothelial surface emerged. The general finding was that as the thickness of the stria vascularis increases, the extent of the pathological

findings, concerning the basal cells, made in connection with atoxyl treatment (Kichuchi & Hilding, 1965; Hilding, 1967) reported that the mitochondria, lysosomes, Golgi complexes and endoplasmic reticulum are seen, within normal range, in the stria vascularis up to 4 weeks of age in the white mouse. However, these structures started to become less numerous, and degenerative changes appeared. Leonard et al. (1967) studied the effects of damage to the stria vascularis of Corti and the stria vascularis, on endolymphatic cationic changes due to treatment with arsacetin. Arsacetin was chosen in view of its established ability to damage both the stria vascularis and the organ of Corti by altering the components of the scala media. Depression of cochlear microphonics (CM) was observed in many animals. The histopathological damage of the organ of Corti was abnormal in instances in which the Preyer's reflex or the acoustic reflex were altered. In animals with depression of endolymphatic, direct-current potential, light microscopic investigation showed that the stria vascularis appeared to have been damaged. In the total histopathological study, isolated instances of damage to the stria vascularis alone could be observed. Tasaki & Katsuragawa (1959) assumed that cochlear microphonics (CM) originate from the hair cells of the organ of Corti, and that the endolymphatic, direct-current potential (EP) originates from the stria vascularis. Investigations by Matschinsky & Thalmann (1967) and by Kuypers (1969) have clearly shown that the marginal cells are involved in the regulation of sodium from the endolymph and are responsible for emitting potassium into this fluid, thereby restoring and maintaining the important electrolyte composition of the endolymph. Disturbance of the metabolism of the marginal cells, which are assumed to be engaged in the transport (Spoendlin, 1967) must interfere with endolymph production and to a certain degree, also with reabsorption. The subsequent degeneration of the marginal

cells, resulting in their rejection, will thus block the endolymph circulation, and the electrolyte balance will collapse. This must interfere with the position of Reissner's membrane, which becomes depressed. A simple rupture at some point cannot result in the general depression of the whole membrane in all its coils. It is apparent that a passage of fluid through Reissner's membrane is not sufficient to allow for a normal location of this membrane. Lawrence (1964) established that perforation of Reissner's membrane at single points causes no damage to the stria vascularis, but the perilymph is absorbed at the local level around the perforation. However, damage was noted in the cells, on the basilar membrane, nearest to the perforation.

As expected, primary lesions of the stria, caused by a substance reaching the stria, might be supposed to appear via the blood stream equally distributed in all turns of the cochlea. However, in several guinea pigs the severest changes were found in the apical coils, whereas the basal turn, although damaged, was not so severely affected. Similar findings were described by Kimura & Perlman (1958) and by Bernstein & Silverstein (1966) by the obstruction of labyrinthine vessels. Kimura & Perlman (1958) found that after arterial obstruction the stria vascularis appeared to become partly detached from the spiral ligament as one unit without edema, hemorrhage or dilation of the vessels. After venous obstruction the stria did not become partly detached but edema, vascular dilation, and fragmentation occurred. The stria vascularis finally disappeared and was replaced by low flat epithelium. The stria in the region adjacent to the round window changed more slowly. The position of Reissner's membrane remained unchanged throughout. However, Bernstein & Silverstein (1966) found a collapse of Reissner's membrane, especially in the apical turns.

Strial atrophy of the ageing ear is well documented by Schuknecht (1955) and by Schuknecht & Igarashi (1964). It is char-

acterized by degenerative changes, however, which begin at the basal end of the cochlear duct and proceed towards the apex, affecting almost equally and simultaneously the various structures within the duct

The finding of an intercellular space sometimes around the blood vessels in the stria vascularis, in connection with atoxyl treatment, is rather obscure. Spoendlin (1967) summarized the general opinion concerning the stria vascularis, and stated that such findings are usually regarded as due to fixation artefacts. There was no such space, however, surrounding the blood vessels in the control material, although it was treated in the same way in the fixation fluid. The term intercellular space is, to some extent, misleading, because arsenicals in general cause damage to blood vessels (Osol & Pratt, 1973). Consequently, the blood vessel is surrounded by blood colloids and electrolytes, hereby causing separation of the marginal-cell extensions from the capillary wall. The intercellular space would, therefore, be the result of increased capillary permeability.

The changes found in the stria vascularis indicate that the stria vascularis is one primary site of action of atoxyl. A degeneration of the stria independent of reason for the degeneration is invariably followed by hair cell damage. The objective of further studies on the stria will be to correlate stria damage with hair cell degeneration in various parts of the cochlea and its physiological significance.

### ACKNOWLEDGEMENT

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### ZUSAMMENFASSUNG

Die Absicht der vorliegenden Untersuchung ist es, auf ultrastrukturellem Niveau die morphologische Degeneration der Stria vascularis nach experimenteller Schädigung darzustellen. Die akute Schädigung nach akuter Atoxyltoxikation tritt nach ca. 12 Stunden auf und beginnt als Degeneration der marginalen und intermediären Zellen

während die basalen Zellen im wesentlichen bleiben. Die stark geschädigten marginalen oder intermediären Zellen können von der Stria vascularis in den endolymphatischen Raum abgestoßen werden. In solchen Fällen bekleiden die basalen Oberflächen zur Endolymph wenigstens bis selten vorkommt. Nach anfänglicher leichter der Reissner'schen Membran kommt es zu Senkung und in manchen Fällen zu einem Vorwölben der Membran, wobei die Reissner'sche Membran gegen das Cortische Organ gefächelt ist. Nur in seltenen Fällen berührt die Reissner'sche Membran die striale Oberfläche. Extension der Mitochondrien mit Bildung von chondrialen Einschlusskörperchen ist ein frühes Befund des Schädigungsmusters.

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## OPTOKINETIC NYSTAGMUS AFTER CEREBELLAR UVULONODULECTOMY IN SQUIRREL MONKEYS

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**Abstract** After cerebellar uvulonodectomy in squirrel monkeys (*Saimiri sciureus*) a change occurred in the optokinetic nystagmus in that there was a slow phase eye speed decline at the stimulus speeds above 90°/sec. The difference was statistically significant when pre and post ablative comparisons were made. This result supports the usefulness of the optokinetic nystagmus examination to diagnose conditions which involve posterior inferior vermis of vestibulo cerebellum. The optokinetic after nystagmus depicted a similar decline postoperatively

based upon both clinical and laboratory observations.

Cogan (1956) previously pointed out the experimental results of the optokinetic nystagmus in animals (such as rabbit) applied to humans only with reservation because of the many differences which exist between the ocular apparatus of man and lower vertebrate experimental animals. Therefore, it is advantageous to study human primates whose ocular system resembles man's for this kind of experimental work. Komatsuzaki et al (1969) reported that the optokinetic nystagmus of the squirrel monkey appeared to be similar to that of man although optokinetic after nystagmus was more prominent in the monkey.

The purpose of the present study was to investigate the change in optokinetic nystagmus following partial ablation of the vermis of the cerebellum (cerebellar uvulonodectomy) in the squirrel monkeys. The information obtained will relate to the clinical diseases involving this particular region.

As mentioned in Adler's textbook (1959), the optokinetic (railroad) nystagmus was studied and used clinically by Barany in 1920 (published in 1921-22). He observed the optokinetic nystagmus (clear pursuit movement) when a black striped roll was rotated in front of an infant's eyes, even a few hours after his birth. Also, he saw a few cases of damage to the frontal area and had an impression that the optokinetic nystagmus was limited in its quick phase.

Presently the optokinetic nystagmus examination is widely used in clinics and this test is considered to be useful especially for the diagnosis of central nervous system disease.

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### SUBJECTS

Eleven squirrel monkeys (*Saimiri sciureus*) were used for the present study. The subjects were healthy young adults about 2 years old.

randomly selected from several different groups (with no preference as to their sex). The subjects were well acclimated to our laboratory conditions prior to the initiation of the experimental procedures.

## PROCEDURE

A squirrel monkey subject was restrained without anesthesia. Application of pressure to the neck region was avoided. Then, the monkey was placed at the center of a 60 cm diameter illuminated white cylinder. The cylinder, which can be rotated by a motor, has 16 equally spaced black stripes that are 1.5 cm wide and 1.5 cm apart. Subdermal platinum needle electrodes were implanted at the outer canthi of the eyes to record the horizontal eye movements. The optokinetic stimulus speed used was 200°/sec with 1°/sec<sup>2</sup> constant angular acceleration. When the stimulus speed reached 200°/sec, the illumination was simultaneously eliminated in order to record the optokinetic after nystagmus.

External stimuli (a remote operated tail clipper and a loud noise applied from a horn) were given to the subject randomly every 2-3 days during the entire optokinetic nystagmus and optokinetic after nystagmus period. Amphetamine, which is a reticular activating system stimulant, was not used in this series of experiments. Both clockwise and counter-clockwise stimulations were used.

Pre-operative optokinetic nystagmus measurements were repeatedly performed with an interval of about 7 days (Pre-Op). Upon acquisition of baseline data, the monkeys were anesthetized with sodium barbital and the area of the cerebellar vermis and nodulus (Larsell's IX and X) were approached through the posterior cranial fossa. Uvulonodectomy was performed by gentle aspiration in 7 monkeys, while the other 4 monkeys were used for sham control operation. In the control group the posterior surface of the cerebellar vermis was exposed but no ablation was performed.

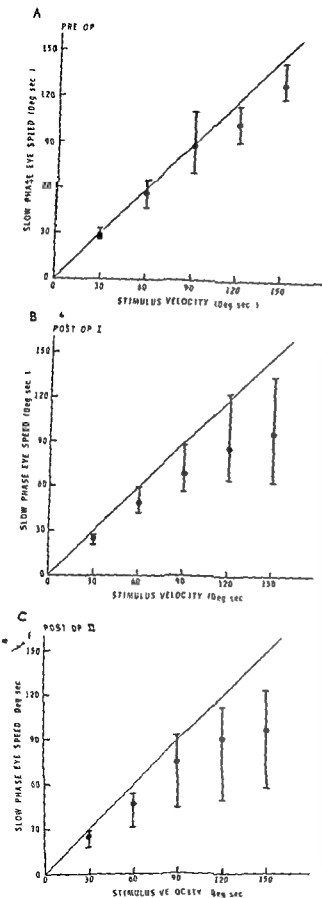
The post-operative optokinetic nystagmus was recorded on the fifth post-operative day (Post Op-I). Later, the examination was repeated after the locomotor equilibrium function (measured by the squirrel monkey tail test) regained the pre-operative level (Post Op-II), the average of 88 post-operative days (the latest test date was 114 days post-operation).

The slow and quick phase eye speeds of optokinetic nystagmus at the stimulus speeds of 30°/sec, 60°/sec, 90°/sec, 120°/sec, and 150°/sec were analysed. All basic numerical values were obtained by averaging three typical nystagmic beats at each level of these stimulus speeds. The numerical values obtained by both clockwise and counter-clockwise stimulations (which showed no distinctive asymmetry) were averaged. The slow and quick phase eye speeds of optokinetic after-nystagmus were obtained by averaging five typical nystagmic beats immediately following the cessation of the optokinetic stimulus.

At the end of the experiment, all subjects were sacrificed in order to morphologically confirm the extent of lesions.

## RESULTS

The slow phase eye-speed of the pre-operative status (Pre-Op) (average of repeated measures), early post-operative status (Post Op I), and the late post-operative status (Post-Op II), were displayed in Fig. 1 A-C. The results were statistically studied at the 30°/sec, 60°/sec, 90°/sec, 120°/sec, and 150°/sec levels of optokinetic stimulus speed, by Duncan's multiple range test. Both Pre Op vs Post-Op I comparison and Pre-Op-II comparisons exhibited statistically significant differences at 90°/sec, 120°/sec, and 150°/sec stimulus speed points, while the comparisons at 30°/sec, and 60°/sec did not show any significant differences (Table I). A comparison of average graphical displays (Fig. 1 A-C) however exhibited a slight lowering of adaptation limit



**Table 1 Optokinetic nystagmus Slow eye speed (post uvulonodectomy)**

Statistics by Duncan's Multiple Range Test  
S Significant difference NS Non-significant  
n=7

	Pre-op	Post-op I	Post-op II
30°	NS	NS	NS
60°	NS	NS	NS
90°	S	S	NS
120°	S	S	NS
150°	S	S	NS

(point of non linearity) after uvulonodectomy. Post operative increase of inter-subject variance was also noticed only at stimulus speeds above 90°/sec. Even though a recovery tendency was noticed when 1 B and 1 C are compared, statistical comparison between Post Op I and Post Op II showed no significant difference (Table 1).

The overall comparisons of quick phase speed between three stages, performed by identical analysis failed to depict any significant difference statistically, even shortly after uvulonodectomy. Dysrhythmia or misinversion were not found. Irregular saccadic movement was seen in only one out of five cases. Amplitude was not consistently post-operatively a slight tendency of amplitude increase was noticed. Frequency generally decreased.

**Fig. 1 (A)** This figure displays the slow phase eye speed at 30°/sec, 60°/sec, 90°/sec, 120°/sec and 150°/sec stimulus velocity points before the cerebellar uvulonodectomy (n=7). The vertical axis indicates the slow phase eye speed and the abscissa represents the optokinetic stimulus speed. Vertical bars indicate the range of obtained individual values. **(B)** The slow phase eye speeds shortly after the operation. **(C)** The slow phase eye speeds long after the operation. Locomotor dyssequilibrium was corrected by this time.

sham control subjects demonstrated a decrease in all in any of the optokinetic stimulus parameters even shortly after uvulonodectomy. On the contrary, the average result from these four monkeys demonstrated a slight response increase after examining optokinetic after nystagmus, the slow phase eye speed depicted a statistically significant difference only between Pre- and Post Op I comparison. The comparison of the quick phase eye speed failed to show a significant difference among different comparisons. The surgical lesions in all seven experimental animals used for the analysis were properly placed (not involving neighboring areas), even though the extent of lesions was slightly varied. Three other subjects with inadequate lesions were not included in the present study.

## DISCUSSION

The usefulness of the optokinetic nystagmus examination as a diagnostic tool for central vestibular system disease was emphasized by Kornhuber (1964). Ino (1970) reported that the pathological findings of horizontal optokinetic nystagmus are frequently observed in cases with central nervous system disease, especially those involving the brain stem. According to his data, more than 50% of cases with central nervous system lesions showed some abnormality of optokinetic nystagmus. The present experimental results confirm the post uvulonodectomy decline of optokinetic nystagmus reported in the previous study (Igarashi et al., 1973) was accurate. The fastigial nuclei project to the regions of the pontine reticular formation near where horizontal eye movement are believed to be initiated and stimulation of these nuclei in the horizontal eye movement (Cohen & Stein, 1972). In different animal species it has been shown that stimulation of the fastigial lobe or of the vermis which overlies the fastigial nuclei mainly induces horizontal eye movement. The results of the present experi-

ment ablating uvula and nodulus (vestibulo-cerebellum) which overlie the fastigial nuclei, indicated change in the slow phase eye speed of the horizontal optokinetic nystagmus in squirrel monkeys.

The classical Dow's study in the monkey (Dow & Moruzzi, 1958) concluded that the nodulus as well as the uvula sent fibers to all four main vestibular nuclei on the homolateral side. According to Brodal (1969), a subsequent study of Angaut & Brodal in 1967 confirmed this concept, also it seems likely that the influence on nystagmus are mediated by the efferent fibers from the nodulus and uvula to the vestibular nuclei. Thus the ablation of the muscle proprioceptor-cerebellum oculomotor vestibular nuclei complex and this connection must have an influence on the optic-oculomotor reflex pathway through the neural pathway between the vestibular nuclei and the oculomotor nucleus (Fukuda et al., 1972). Eye muscle proprioceptor cerebellum-oculomotor nucleus pathway must also be involved by uvulonodectomy.

According to Komatsuzaki et al. (1969), the slow phase velocity of the optokinetic nystagmus was most closely related to the optokinetic stimulus speed in rhesus monkeys. Based upon this information in the present experiment the slow phase eye speed of horizontal optokinetic nystagmus was analysed in squirrel monkeys. Cohen et al. (1973) found that the slow phase eye speed of optokinetic nystagmus returned to the original values 10 months after the labyrinthectomy. There may be a possibility that similar recovery may have occurred if the animals in the present study had been permitted to survive longer.

Uvulonodectomy resulted in optokinetic hypo-nystagmus at high stimulus speeds, therefore, the present experimental results may be extrapolated to the acute conditions such as whip lash automobile injury, sudden vascular occlusion which involves the region, and certain forms of war injury, etc. On the other hand chronic diseases such as slowly growing tumor or infection, etc. may not



necessarily depict this kind of alteration in optokinetic nystagmus. According to Tokita's report, however, 5 cerebellar tumor cases (medulloblastoma 2, glioblastoma 1, hemangioblastoma 1, other 1) exhibited low slow phase eye speed and irregular (step like) wave form (Tokita, 1973).

The presence of post-uvulonodectomy spontaneous nystagmus was very inconsistent. Generally the spontaneous nystagmus had a more vertical component than horizontal one. Furthermore, even though the presence of spontaneous nystagmus enhances optokinetic nystagmus (when it has a horizontal component), the post-uvulonodectomy optokinetic nystagmus showed a statistically significant decline when compared with that of the pre-operative condition.

When the pre-operative measurements and the post-operative measurements were compared in control subjects, a slight response increase of the slow-phase eye speed of optokinetic nystagmus was found, even though the testing was performed only infrequently. Therefore, the post-uvulonodectomy optokinetic nystagmus 'decline' represented true ablation effects and not artefactual results.

Significant post-uvulonodectomy optokinetic hypostasy was only found when the stimulus speed was higher than 90°/sec, thus this result confirms the importance of the high speed optokinetic stimulus. The post-uvulonodectomy decline of optokinetic nystagmus in this experiment confirms the importance of uvulonodular input to the optically evoked oculomotor response, which ever neural channels are involved. The similar reduction of optokinetic after-nystagmus following uvulonodectomy probably indicates that the optokinetic after-nystagmus is a reflexory response of the optokinetic nystagmus.

## ZUSAMMENFASSUNG

Nach Entfernung der zerebellaren Uvulonodulektomie beim Totenkopfpaffen (*Samus Scireus*) erfolgte eine

Veränderung im optokinetischen Nystagmus, eine Verminderung der langsamen Augenbewegung bei einer Anreizgeschwindigkeit von über 90°/sec. Unterschied war statistisch bedeutend wenn nach der Operation Vergleiche angestellt wurden. Resultat bestätigt wie nützlich die Uvulonodulektomie ist um gewisse Läsionen zu bestimmen die das hintere unten gelegene Zapfen im Vorhof des Kleinhirns betreffen. Optokinetische Nachnystagmus zeigte eine Abnahme nach der Operation.

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## PLANAR RELATIONSHIPS OF THE SEMICIRCULAR CANALS IN MAN

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Principal-component analyses were determined of points measured from the dissected bony floor of ten human skulls resulting in planar equations for each of the six semicircular canals. Following angles were calculated between the ipsilateral canal and between opposite synergistically acting canal and between each canal and the Reid stereotaxic Results indicated that pairs of ipsilateral canals were nearly perpendicular with the exception of the angle between the anterior and horizontal canal. Pairs of contralateral synergistic canals formed angles of 19° between right and left horizontal planes and 23-24° between vertical canal pairs. Horizontal canals formed an angle of 25° with the Reid vertical plane. Mathematical equations of the semicircular canals were used to predict the optimal head position for rotational and caloric stimulation.

Extensive number of studies have used human subjects to study the stimulus-response characteristics of the semicircular canals. In most of these, angular acceleration was produced by rotation about the major head axes

(Melvill Jones et al, 1964, Clark & Stewart, 1968, Oosterveld, 1970, Guedry et al, 1971, also see review by Clark, 1967) producing a combined stimulation of several pairs of canals. In other studies, however, subjects were positioned for the purpose of stimulating a single pair of vertical canals (Van Der Vis, 1958, Decker, 1969). Positioning was based on the assumption that the vertical canals were oriented perpendicular to the horizontal canal and formed 45° angles with the sagittal plane. For the horizontal canals, and using angular acceleration in the earth horizontal plane, descriptions of head position have ranged from a 30° (Van Egmond et al, 1949, Fitzgerald & Hallpike, 1942) to a 20-25° bent forward position (Graybiel et al, 1948). Other investigators have not described their method of positioning the head but have stated that the horizontal canals were aligned in the plane of rotation (Brown & Crampton, 1964).

Canal stimulation experiments using subjects in a variety of head positions raise the questions of which canals are activated by the stimulus and to what degree. It has been known since the time of Ewald that a single canal will be maximally stimulated when rotated in its plane and minimally affected when rotated in a plane perpendicular to the canal

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plane. It can be concluded that to stimulate individual canals or pairs of canals properly and to control the amount of stimulation to other canal pairs, the position of the canal planes must be known.

A complete description of the human semicircular canals in relationship to the stereotaxic reference system and the internal angular relationships between canal planes has not appeared in the literature. Previous studies have been mainly limited to an angular description of a single canal. Spiegel & Sommer (1947) describe the horizontal canal as being elevated  $15-30^\circ$  above the horizontal plane while Girard (1923) gives a value of  $25^\circ \pm 7^\circ$  for the same angle. Other workers, however, have reported smaller angles formed by the horizontal canal with the Frankfurt horizontal plane. For example, Kudo (1965) reported  $15^\circ 87'$  (sic) and Nishimura (1930)  $12.2^\circ$ .

A few authors have described the angular relationships of the vertical canals. Colosi & Giannardi (1968) examined the angles formed by the vertical canals and the vertical stereotaxic planes in X-ray material. These angles were also described by Spiegel & Sommer (1947) and by Minckler (1968).

It is clear from this brief review of the literature that more information is required to define more precisely the position of the semicircular canal planes with respect to skull.

This report is concerned with a mathematical description of the human semicircular canal planes with respect to each other and to the stereotaxic coordinate system.

## PROCEDURES

A description of the position of the semicircular canals requires a convenient external frame of reference. For this reason the Reid stereotaxic coordinate system was employed. This system defines the horizontal plane as one passing through the inferior margin of the orbits (orbitale) and the center points of the two external auditory canals. The Reid

stereotaxic frontal and sagittal planes are perpendicular to the horizontal plane and pass through the center of the external canal and mid sagittal suture. Mathematical conventions of Hixson et al (1966) to define the system were used throughout this study. In comparison, measurements of the horizontal plane (Ohr Auger) were taken. This reference system differs from Reid in that the horizontal plane and subsequently the vertical planes, are defined by the above orbital points and the margin of the external auditory canal.

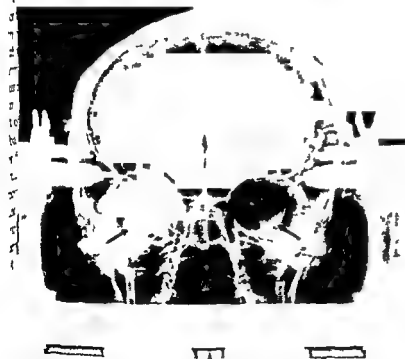
Material consisted of ten racially mixed man skulls (Carolina Biological Supply Company, Burlington, N.C.) which were free of pathology or bone deformities. Skulls were secured to a Talairach human stereotaxic instrument (Schaltenbrand & Bailey) with two ear bars placed in the center of the external auditory canal and two eye bars placed in the superior orbit. The eye bars made contact with the inferior orbital rims. These four craniometric points were aligned with the horizontal skull plane with the stereotaxic frame and measuring instrument. The skull was then secured with four pins (A and B). Micrometer adjustments of the instrument permitted accurate realignment of the skull after removal of the eye and ear bars and facilitated the removal of the skull for dissection.

Exposure of the semicircular canals for measurement required the removal of the external ear. The skin of the external ear of the mastoid process and middle ear structures. Each canal was exposed by making an incision about its curvature from the ampulla to the body of the vestibule. After dissection, each skull was then remounted in the stereotaxic instrument.

To insure proper alignment of the skull in relation to the stereotaxic frame and measuring device, both anterior-posterior (A-P) and lateral X-rays were taken of each skull (A and B). Tube to plate distance was 50 cm, producing a magnification of 5%. The X-ray tube was aligned by means of a spirit level with a front to back target separation.



A



B

A Lateral X ray of a skull fixed in the stereotaxic frame. The temporal bones have been dissected, altering the bony landmarks. The measuring device (not shown) is attached to the broad horizontal bar at the posterior of the picture. The craniometrically defined Reid line (black line in figure) is defined by black dots placed

on the inferior surface of orbits and at center of external auditory canal. Arrows indicate markers made of metal particles placed at the junction of common crus and vestibule of both labyrinths. In this preparation, springs support the mandible and a latch holds the convexity of the skull. B Anterior-posterior view of skull shown in A.



Fig 2 A A right pos view of canal d ssection illustrates the perspective of 2 B and of 3 A B C

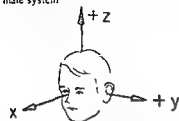
Fig 2 B Higher magnification of semicircular canal d ssection

250 mm. Deviations of less than  $0.5^\circ$  from a true lateral and A-P view (at the level of Reid Horizontal Plane) were produced by this alignment procedure.

With each skull mounted in the frame planes of excursion of an x-ray manipulator (Prior Farmingdale N.Y.) aligned parallel to the stereotaxic planes.

# **Fig. 1. Mean planar equations for each semicircular canal plane defined in the Reid ototoxic system**

in figure indicates the axes and polarity of the Reid ototoxic system



Semicircular Canal	Planar Equation
Horizontal canal	$365x + 158y + 905z = 0$
Anterior canal	$652x + 753y + 017z = 0$
Posterior canal	$757x + 561y + 320z = 0$
Horizontal canal	$365x + 158y - 905z = 0$
Anterior canal	$652x + 753y - 017z = 0$
Posterior canal	$757x + 561y + 320z = 0$

three dimensional coordinates (to the nearest 0.1 mm) of a series of approximately 100 spaced points along each semicircular canal were measured by means of a sharp needle attached to the micromanipulator. Under magnification from 30 to 103 points were marked on each canal, the difference in length being related to differences in canal diameter (see Fig. 2). Points were taken from the common crus to the ampulla of each vertical canal and from the body of the vestibule to the ampulla in the horizontal canals. Data were taken from the medial most portion of the bony vertical canals and from the anterior most portion of the bony horizontal canals. Points within the ampullary regions

were not included in subsequent calculations.

Translational calculations were performed on the original data to refer each point to the stereotaxic zero. Each point on a canal thus represented a three-dimensional vector in the Reid stereotaxic coordinate system. Data points belonging to each canal were subjected to computer analysis using both a principal component and a multiple regression technique (Blanks et al., 1972). Both methods gave the statistically weighted, best fitting planar equation passing through all points for each canal and provided virtually identical results. Only those values derived from principal component analysis (Thurstone, 1957) will be reported here. This technique provides the coefficients  $A$ ,  $B$  and  $C$  of the generalized equation for a plane  $Ax + By + Cz + D = 0$ . The  $D$  term has been set to zero. Thus the equations presented are for planes parallel to the nominated canal plane passing through the origin. This has no effect on the computation of angles between planes. Once derived, planar equations for each canal served as input to several computer programs which provided angular interrelationships between the canal planes.

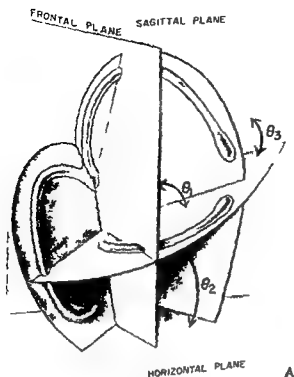
## RESULTS

Craniometric measurements on the skull material revealed a mixture of craniometric shapes. The cephalic index (CI) defined as the ratio of the maximum cranial breadth and the

## **2.1. Angles in degrees between pairs of human semicircular canal planes**

Standard deviation (S.D.) and confidence limits for angles between ipsilateral canal planes represent combined and left values ( $N = 20$ ). Angles between synergistic canal pairs were considered as unique values and were not combined ( $N = 10$ ).

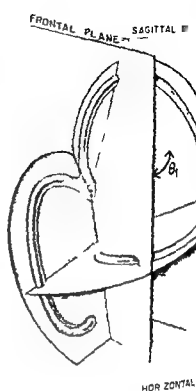
Pair of canal planes	N	Mean $\pm$ S.D.	95% confidence limits	
			Lower	Upper
Horizontal-Anterior	20	111.76 $\pm$ 7.55	108.23	115.30
Horizontal-Posterior	20	86.16 $\pm$ 4.72	83.95	88.38
Horizontal-Right horizontal	20	95.75 $\pm$ 4.66	93.56	97.93
Anterior-Right posterior	10	114.56 $\pm$ 7.19	109.41	119.70
Posterior-Right anterior	10	23.73 $\pm$ 6.71	18.93	28.53
Horizontal-Right horizontal	10	19.82 $\pm$ 14.93	9.14	30.50



ANGLES IN DEGREES BETWEEN HUMAN CANAL PLANES AND THE REID STEREOTAXIC PLANES

Angle between the Horizontal Canal Plane & Stereotaxic	Mean + SD	N	95% Confidence Limits	
			Lower	Upper
Frontal plane $\theta_1$	68.60 + 5.86	20	65.85	71.34
Horizontal plane $\theta_2$	25.12 + 5.62	20	22.49	27.75
Sagittal plane $\theta_3$	99.10 + 8.76	20	95.00	103.20

Fig 3 A-C Location of the angles ( $\theta_1$ ,  $\theta_2$ ,  $\theta_3$ ) between the semicircular canal and stereotaxic planes from vector or product calculations. Values represent mean from pooled right and left sides ( $N=20$ ). The right canal



ANGLES IN DEGREES BETWEEN HUMAN CANAL PLANES AND THE REID STEREOTAXIC PLANES

Angle between the Anterior Canal Plane & Stereotaxic	Mean + SD	N
Frontal plane $\theta_1$	49.32 + 4.79	20
Horizontal plane $\theta_2$	89.00 + 4.94	20
Sagittal plane $\theta_3$	41.10 + 4.82	20

planes are illustrated. Location for values on mirror image locations. Angular values and the horizontal canal are shown in A for anterior canal in B and for the posterior canal in C.

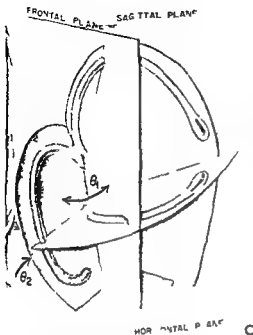
maximum cranial length ranged from 70 to 77.5. Seven skulls were of the dolichocephalic or long skull type ( $70 \leq CI \leq 74.9$ ), and three were of the mesocephalic type ( $75 \leq CI \leq 79.9$ ) (Schaltenbrand & Bailey, 1959).

### Planar equations

Planar equations representing the average equation for each canal plane defined in the Reid stereotaxic reference system are given in Table I. Equations were derived by averaging the values from each of the ten skulls. The insert in Table I depicts the Reid reference axes.

The magnitude of the  $x$ ,  $y$ , and  $z$  components for each canal is important in predicting canal response to roll, pitch and yaw accelerations respectively. It will be seen from Table I that each canal has a significant component into the roll plane (magnitude of the  $x$  component) and into the pitch plane (magnitude of the  $y$  component). In the horizontal canal (0°), the posterior canal (0°/120°) would be affected by angular acceleration while the anterior canal (0°/17°) would be virtually unaffected.

While a vectorial representation of the



ANGLES IN DEGREES BETWEEN HUMAN CANAL PLANES AND THE REID STEREOTAXIC PLANES

between the tor Canal Stereotaxic	Mean $\pm$ SD	n	95% Confidence Limits	
			Lower	Upper
plane $\theta_1$	40.80 $\pm$ 4.97	20	38.47	43.13
tal plane $\theta_2$	71.36 $\pm$ 6.20	20	68.45	74.26
plane $\theta_3$	55.84 $\pm$ 3.95	20	53.99	57.69

is useful for kinematic analysis of the  
the angular relationships are also help  
three sets of angles were calculated (1)  
formed between pairs of canal planes  
ose formed between canal planes and the  
otaxic planes and (3) those which enable  
ptimal positioning of single canals for ro-  
tal and caloric stimulation

#### Angles between pairs of canal planes

Table II shows the calculated angles between  
specific pairs of canal planes. Only small dif-  
ferences were found between the correspond-  
ing canals on the right and left sides and there-  
fore the left and right data were combined to  
represent a population of 20. This was not possible  
for angles between pairs of contralateral

synergistically acting canal planes ( $n=10$ ). It  
will be noted that ipsilateral canal planes do  
not form true right angles with adjacent canals.  
The more nearly perpendicular pairs were the  
anterior and posterior canals (86.16°) and the  
posterior and horizontal canals (95.75°). A  
much greater degree of non-perpendicularity  
was found between the horizontal and anterior  
canal planes (111.76°). The fact that 95% con-  
fidence limits on angles between ipsilateral  
canal planes did not include 90° indicates these  
canals are not orthogonal.

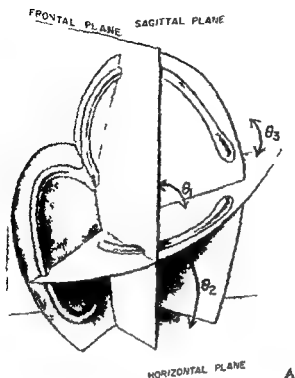
Table II also indicates the degree of co-  
planarity found between pairs of synergistic  
canal planes. Mean angles between the left  
anterior and right posterior canals and be-  
tween the left posterior and right anterior were  
24.56° (SD = 7.19) and 23.73° (SD = 6.71).  
Angular deviation between contralateral hori-  
zontal canals was more variable (SD = 14.93°)  
but of similar magnitude (19.82°).

#### Relationship between the canal planes and the stereotaxic planes

The stereotaxic orientation of the right canal  
planes and the angles made with the stereo-  
taxic planes are given in Figs 3 A, B, C. Figs 2 A and B illustrate the perspective from  
which Figs 3 A, B and C were drawn. The  
right horizontal canal (Fig 3 A) forms an  
angle of 25.12° with the Reid horizontal plane  
and is tipped slightly with respect to the  
sagittal plane (higher medially, lower laterally)  
resulting in a mean angle of 99.10° with the  
sagittal plane. The lateral tilt in both hori-  
zontal canals results in the degree of non-  
coplanarity noted earlier (19.82° between right  
and left horizontal canal planes) in Table II.  
Most (14/20) horizontal canal pairs showed  
this lateral inclination while some (7/20) were  
essentially perpendicular to the sagittal plane  
and others (4/20) were tipped a few degrees in  
an opposite fashion (higher laterally, lower  
medially).

The orientation of the vertical canals with  
respect to the sagittal plane was less variable  
with the anterior canal forming an angle less





ANGLES IN DEGREES BETWEEN HUMAN CANAL PLANES AND THE REID STEREOTAXIC PLANES

Angle between the Horizontal Canal Plane & Stereotaxic	Mean $\pm$ SD	N	95% Confidence Limits	
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ANGLES IN DEGREE AND THE R<sup>2</sup>

Angle between the Anterior Canal Plane & Stereotaxic	$r^2$
Frontal plane $\theta_1$	.49
Horizontal plane $\theta_2$	.83
Sagittal plane $\theta_3$	.41

planes are illustrated local mirror image locations. A the horizontal canal are sh B and for the posterior can

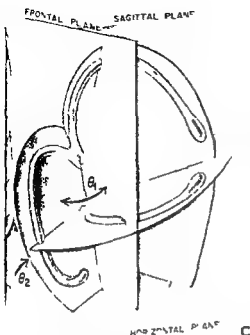
maximum cranial length, ranged from 70 to 77.5. Seven skulls were of the dolichocephalic or long skull type ( $70 \leq CI \leq 74.9$ ), and three were of the mesocephalic type ( $75 \leq CI \leq 79.9$ ) (Schaltenbrand & Bailey, 1959).

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The magnitude of the response for each canal is important for canal response to roll and yaw accelerations respectively. Table I that each canal has a component into the roll plane component) and into the yaw plane component (magnitude of the y component) the horizontal component of the posterior canal (0.320) affected by angular acceleration of the anterior canal (0.017) would be affected.

While a vectorial representation



ANGLES IN DEGREES BETWEEN HUMAN CANAL PLANES AND THE REID STEREOTAXIC PLANES

Angles between the Canal and Stereotaxic		Mean $\pm$ SD	N	95% Confidence Limits	
				Lower	Upper
Horizontal plane	$\theta_1$	40.80 $\pm$ 4.97	20	38.47	43.13
Sagittal plane	$\theta_2$	71.36 $\pm$ 6.20	20	68.45	74.26
Vertical plane	$\theta_3$	55.84 $\pm$ 3.95	20	53.99	57.69

When useful for kinematic analysis of the ear, the angular relationships are also helpful. Three sets of angles were calculated: (1) those formed between pairs of canal planes, (2) those formed between canal planes and the stereotaxic planes, and (3) those which enable optimal positioning of single canals for rotational and caloric stimulation.

#### Angles between pairs of canal planes

Table II shows the calculated angles between the pairs of canal planes. Only small differences were found between the corresponding canals on the right and left sides, and therefore the left and right data were combined to form a population of 20. This was not possible for angles between pairs of contralateral

synergistically acting canal planes ( $n=10$ ). It will be noted that ipsilateral canal planes do not form true right angles with adjacent canals. The more nearly perpendicular pairs were the anterior and posterior canals (86.16°) and the posterior and horizontal canals (95.75°). A much greater degree of non-perpendicularity was found between the horizontal and anterior canal planes (111.76°). The fact that 95% confidence limits on angles between ipsilateral canal planes did not include 90° indicates these canals are not orthogonal.

Table II also indicates the degree of coplanarity found between pairs of synergistic canal planes. Mean angles between the left anterior and right posterior canals and between the left posterior and right anterior were 24.56° (SD = 7.19) and 23.73° (SD = 6.71). Angular deviation between contralateral horizontal canals was more variable (SD = 14.93) but of similar magnitude (19.82°).

#### Relationship between the canal planes and the stereotaxic planes

The stereotaxic orientation of the right canal planes and the angles made with the stereotaxic planes are given in Figs 3 A, B, C. Figs 2 A and B illustrate the perspective from which Figs 3 A, B and C were drawn. The right horizontal canal (Fig 3 A) forms an angle of 25.12° with the Reid horizontal plane and is tipped slightly with respect to the sagittal plane (higher medially, lower laterally) resulting in a mean angle of 99.10° with the sagittal plane. The lateral tilt in both horizontal canals results in the degree of non-coplanarity noted earlier (19.82° between right and left horizontal canal planes) in Table II. Most (14/20) horizontal canal pairs showed this lateral inclination, while some (2/20) were essentially perpendicular to the sagittal plane and others (4/20) were tipped a few degrees in an opposite fashion (higher laterally, lower medially).

The orientation of the vertical canals with respect to the sagittal plane was less variable, with the anterior canal forming an angle less

Table III *Naso-occipital and lateral inclination for each canal plane (defined in text)*

Values for the right and left sides have not been combined to demonstrate degree of right-left symmetry. A 'Direction' indicates the orientation of the angle OA=open anteriorly, OP=open posteriorly OL=open right

Semicircular canal	N	Naso-occipital inclination			Lateral inclination		
		Mean	S.D.	Direction	Mean	S.D.	Direction
Horizontal							
Left	10	21.44	4.82	O.A	9.43	9.81	O.R
Right	10	22.38	6.95	O.A	10.16	9.27	O.L
Anterior							
Left	10	91.18	7.76	O.P	91.64	6.77	O.L
Right	10	91.31	7.90	O.P	91.26	6.75	O.R
Posterior							
Left	10	68.01	7.98	O.P	61.00	8.04	O.R
Right	10	66.26	7.80	O.P	60.52	9.69	O.L

than 45° (41.10°) (Fig. 3 B) and the posterior canal an angle greater than 45° (55.84°) (Fig. 3 C). This general pattern of orientation for the vertical canals in relation to the sagittal plane agrees with the figures given by Colosi & Giannardi (1968) who measured from X-ray material the angle formed by the anterior canal (35.5–37°) and the posterior canal plane (59.5–61.5°) in relation to the anterior-posterior axis.

#### Right-left symmetry

To demonstrate the degree of right-left symmetry found in the angular relationships in the present material, a pair of 'locating angles' (naso-occipital and lateral inclination angles) are given for each canal which were calculated from the ratio of two directional cosines. That is, given a general canal planar equation defined in the Reid stereotaxic coordinate system (Table I) of the form,  $ax+by+cz=0$ , the naso-occipital inclination is expressed as  $\tan^{-1}(c/a)$  and the lateral inclination as  $\tan^{-1}(c/b)$ . It must be emphasized that these angles represent values projected into a third plane and as such, do not describe the angles between planes as presented earlier (Figs. 3 A, B and C, Table II). These angles are given in Table III.

As shown in Table III, the naso-occipital

inclination of 10 right and 10 left canals was 22.38° and 21.44°. The canal lateral inclination values for canals were 10.16° and 10 left was 9.43°. Close examination of the value III for A-P and lateral inclination indicate only small right-left difference values (1–2°). The absence of large values in mirror image canal pairs and left horizontal canal A-P inclination indicates the absence of data skew may have resulted from skull misorientation in the stereotaxic frame.

#### Angular position of the horizontal semi-circular canal with respect to the Frankfurt horizontal plane

In order to compare the results from the present study with the results of others with respect to the Frankfurt plane, it was necessary to define the angular relationship between the Reid and Frankfurt horizontal planes. Measurements from craniometric and X-rays (Fig. 1 A) indicate a small difference of  $4.43 \pm 1.14^\circ$  between the Reid horizontal plane defined by the center of the external auditory canal and the inferior orbital plane and the Frankfurt horizontal plane defined by the superior margin of the external auditory canal and the same

s Combining the average right-left value for the horizontal canal naso-occipital inclination in the Reid system (Table III) and the angular difference between the two reference planes ( $4.43^\circ$ ), the horizontal canals are inclined  $26.34^\circ$  with respect to the Frankfurt

These observations agree closely with the findings in the literature that the horizontal canals are inclined  $30^\circ$  open anterior to the horizontal plane (Fitzgerald & Hallpike, 1942; El & Sommer, 1947) and the anatomical measurements of  $25^\circ \pm 7^\circ$  by Girard (1923),  $24.96^\circ$  by Fenart & Dardenne (1968),  $27^\circ$  by Perez (1922), and about  $28^\circ$ – $37^\circ$  by calculation of data of Robin et al. (1967).

What smaller values for the A-P inclination of the horizontal canal with respect to the Frankfurt plane were obtained by Kudo (1965) of  $8.7^\circ$  (sic), by Nishimura (1930) of  $12.2^\circ$ , by Giemann (1904) of  $13.03^\circ$ . These smaller values may be due to differences in skull shape. Our samples of skulls were classified as microcephalic and mesocephalic (3) whereas skulls measured by Kudo were brachycephalic in shape. Further information is needed to explain this difference.

## DISCUSSION

Linear measurements on the human semicircular canals in previous studies have varied from measurement of X rays (Colosannardi, 1968; Mincker, 1968) or by direct measurement of a few points on each canal (Kudo, 1965; Girard, 1923). Both techniques provide information on the angular position of the canals, but the degree of accuracy of these techniques and the inability to define the canal plane in a stereotaxic reference frame lessens the value of these results. The method employed in this report, i.e., taking a large number of approximately equally spaced data points per canal (from 30–103) and then defining each canal with a best fitting plane results in greater accuracy.

This permitted a check on craniometric and X-ray determined skull reference planes. Examination of X-ray material revealed a small ( $<2^\circ$ ) misalignment of the Reid planes with the stereotaxic instrument frame in some skulls. This was compensated for in the final calculation of all angles noted in the Results.

One concern with the present technique, as well as with previously mentioned methods of canal measurement, is that less than the entire  $360^\circ$  circumference of the toroid ring was measured. That is, the ring of endolymph which begins in the semicircular canals and is completed through the utricle was not measured. In most cases the arc of bony canal over which data points were taken was about  $174^\circ$ . The degree to which the portion of the endolymph fluid torus lying within the utricle contributes to the biologically active canal plane is uncertain. It can be inferred that if the plane passing through the semicircular canal arc is everywhere continuous with the endolymph channel within the utricle, then the planes measured here will represent the entire toroid. However, if the membranous walls of the utricle distort the endolymph fluid toroid, then some alteration of the functional canal plane would be expected. Careful histological examination of the portion of the canal plane within the vestibule will be important in determining this possibility.

One additional factor in the accuracy of the present technique in determining the position of the biological response plane of the canals is the alignment of the membranous and osseous canals. It will be remembered that the present technique measures the medial most portion of the osseous vertical canals and the superior-most portions of the osseous horizontal canal. However, the biological plane of the canal is determined by the position of the membranous canal. In the human, the membranous canals lie against the walls of the bony canal at the point of maximal osseous canal radius ( $R$ ) (Igarashi, 1966; Curthoys et al., in preparation). This means that the plane of the membranous canal is nearly parallel

Table IV Positioning angles for human semicircular canal stimulation

Semicircular canal plane	Rotational				Caloric	
	Pitch angle		Roll angle		Pitch angle	
	Angle	Direction	Angle	Direction	Angle	Direction
Left horizontal canal	21 91	PND	9 11	RRED	68 09	POD
Left anterior canal	91 25	POD	48 90	RLED	1 25	POD
Left posterior canal	67 14	POD	34 16	RRED	22 86	PND
Right horizontal canal	21 91	PND	9 11	RLED	68 09	POD
Right anterior canal	91 25	POD	48 90	RRED	1 25	POD
Right posterior canal	67 14	POD	34 16	RLED	22 86	PND

bony canal plane and would indicate that the angular measurements in the bony canal planes in the present study would apply also to the membranous canal plane. In view of the large perilymphatic space in the human canal system, the possibility of a systematic deviation of the membranous canal from the bony canal plane cannot be entirely eliminated. The maximal possible angular error in such cases would be related to the ratio of the canal radius of curvature ( $R$ ) and the difference in cross sectional radius between the osseous ( $r_o$ ) and membranous ( $r_m$ ) portions of the canal. Among the three canals in the human, biophysical measurements by Igarashi (1966) predict that the largest angular deviation between the osseous and membranous canal planes could occur in the horizontal canal where the combination of a small radius of curvature ( $R=3.2$  mm) and large perilymphatic space ( $r_o-r_m=0.63$  mm) result in an angle of  $5.4^\circ$  ( $\tan^{-1} [0.63 \text{ mm}/3.2 \text{ mm}]$ ).

The measurement technique employed here on humans has been shown to be a valid way of establishing semicircular canal planes. Planar equations derived by this technique from seven cat skulls (Blanks et al., 1972) have been found to be in close agreement with the physiologically determined response planes of first-order canal neurons in the cat (Estes et al., 1975). Angular differences of from  $5$ – $12^\circ$

were found between anatomically determined canal planes when compared with physiologically determined values.

#### Positioning for vestibular stimulation

The most valuable result of the present study is that a mathematical description of the system enables the orientation of human subjects for more accurate stimulation of individual canal receptors. It should be emphasized that because contralateral synergists are involved (Table I), orientation of the head for stimulation of bilateral synergists will be required. To promise each canal will deviate from the plane of acceleration. Table IV gives the angles for each canal which permit positioning of the head for optimal orientation for rotation or caloric stimulation. The values in this table are calculated using an upright orientation as an initial position (Reid 1964). The horizontal plane parallel to an earth horizontal order and axis about which the head is positioned are important and values are calculated on the basis of first pitch. The first pitch is rotation about the interaural axis which is then defined as rotation about an earth horizontal axis coincident with the sacral axis. Values for rotational stimulation are given in the left columns of Table IV. An example of head positioning for stimulation of the

rior canal is a 67° pitch occiput down  
-b) followed by a 34° roll left ear down  
(D)

From the results of this study the optimal  
position for stimulation of both horizontal  
canals is by an angular acceleration in an earth  
horizontal plane with the head pitched forward  
close down from the Reid horizontal plane  
(above). Because of the noncoplanarity of  
horizontal canals in this position each  
horizontal canal is approximately 9° out of the  
plane of stimulation. Also in this position the  
vertical canals form angles of 75° with the  
plane of stimulation whereas the posterior  
vertical canals are almost exactly perpendicular to the  
plane of stimulation (90.8°). Because the  
vertical canals are not perpendicular to the  
plane of the acceleration, they will both be  
stimulated by an angular acceleration about  
the vertical axis of the magnitude of that applied to the  
horizontal canals ( $\cos[75] = 0.26$ ).

The values given in Table IV are figures for posi-  
tioning the subject for caloric stimulation.  
The calculations were based upon an initial  
head position. A single rotation of 68°  
for the horizontal canal, 10° POD for the  
anterior canal, and 23° POD for the posterior  
vertical canal is required to bring the 'target' canal  
into the earth vertical position. It should be  
noted that the last two positionings still leave  
the horizontal canal angulated from earth hori-  
zontal.

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## ZUSAMMENFASSUNG

Pol-component Analysen wurden an einer Serie von

von Reid berechnet. Die Resultate zeigen, dass Paare von  
ipsilateralen Kanälen nahezu rechtwinklig zueinander  
stehen. Eine Ausnahme bildet der Winkel zwischen dem  
vorderen und horizontalen Kanal (Mittel = 111°).  
Synergistische Kanalpaare bilden einen Winkel von 19°  
zwischen dem rechten und linken horizontalen Kanal und  
von 23-24° zwischen vertikalen Paaren. Der horizontale  
Kanal bildet einen Winkel von 25° mit der Reidschen  
Horizontalebene. Mittels mathematischer Gleichungen  
wurde die optimale Kopfposition für rotationsche und  
kalorische Reizung ermittelt.

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## THE ORIENTATION OF THE SEMICIRCULAR CANALS IN THE GUINEA PIG

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In 10 adult guinea pigs the stereotaxic coordinates of a series of points along each osseous semicircular canal were analysed to yield an equation of that canal in stereotaxic space. Angular relationships among canal planes and between the canal planes and the stereotaxic planes are presented together with the positions of the head for physiological stimulation of a canal or pair of synergistic canals. The planes of semicircular canals in each labyrinth are not perpendicular to one another and the planes of contralateral canals depart from parallelism by about 30°.

In order to specify the mechanical forces in the semicircular canal stimulation it is necessary to have information about, among other things, the fine structure of the canal and the orientation of the canal relative to the direction of the stimulating acceleration. Optimal physiological stimulation occurs when the plane of stimulation is parallel to the plane of rotation of the canal. If the canal is tilted from parallelism the magnitude of the angular acceleration stimulation decreases as a cosine function (Blanks, van Egmond et al, 1952) and other canals are increasingly stimulated. We have developed a technique which determines the

orientation of the semicircular canal planes with reference to external skull landmarks (Blanks et al, 1972, 1975) enabling the head to be positioned to optimally stimulate any canal. This technique has yielded data for the cat and human; the present paper reports comparable information for the guinea pig.

The guinea pig has been used extensively in anatomical and physiological studies of the vestibular system. Its semicircular canals are about the same size as those of the cat and much more easily accessible (Curthoys et al, 1971). Nevertheless there is surprisingly little data concerning the orientation of these canals in the skull. Kristensen (1954) reported that the planes of the horizontal canals are parallel with a plane passing through the external auditory meatus and the supraorbital margins. Wersall (1956) reported that the two horizontal canals are in the same plane and the vertical canals are perpendicular to this plane. He estimated the angle between the ipsilateral anterior and posterior canal planes to be 100°, Bodechtel (1930) has estimated this angle to be 115°. Wersall also reported that the two posterior canals formed an angle of 110° with each other. These results lead to the deduction that both pairs of vertical synergistic canals (the left anterior right posterior and right anterior-

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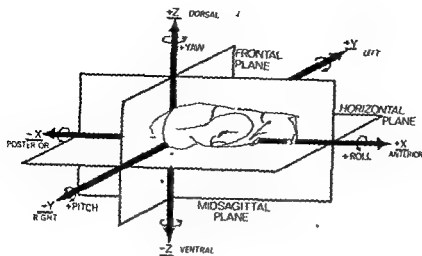


Fig 1 The stereotaxic guinea pig and the conventions used

left posterior) are misaligned from parallelism by about 25°

Because of the need for a more complete and accurate specification of semicircular canal planes in the guinea pig, particularly for skull positioning during angular acceleration stimulation we applied a technique, described in detail elsewhere (Blanks et al, 1972) to determine these planes in the stereotaxic axes system

## METHODS

### Guinea pig stereotaxic planes

In stereotaxic systems the horizontal plane is the most difficult to define for the cat, monkey and human, something akin to the Reid plane is used (the plane joining the centres of the external auditory canals and the inferior margins of the orbits). Definitions of the stereotaxic horizontal plane for rodents are far less uniform (de Groot, 1959, Hurt et al, 1971, König & Klippel, 1963, Luparello, 1967, Luparello et al, 1964, Pellegrino & Cushman, 1967, 1971, Tindal, 1965). Tindal did define the horizontal plane for the guinea pig by the external auditory canals and the inferior margin of medial canthi. However, his animals were so unusually large (700–830 g) that he was able to use a Kopf cat/monkey head holder. The other widely used stereotaxic system for the guinea pig defines the horizontal plane by

means of the heights of the skull surr and 14 mm anterior to the intern (Luparello, 1967). Instead of using these systems we chose to use the Kopf pig head holder with the incisor vertical adjustment set at zero. In this way the horizontal plane becomes the plane through centres of the two external auditory canals the inferior-most portion of the bone posterior to the incisors (i.e. the rostral edge incisor bar). Measurements of a few skull dimensions enabled simple transfer the semicircular canal planes to the plane outlined above. The sagittal plane is perpendicular to the horizontal plane and through the midsagittal suture. The plane is perpendicular to the two others passing through the centres of the external auditory meati. The Hixson, Niven and reia mathematical conventions were (1966) in this system the x axis is the capital axis (anterior positive) the y axis is the interaural axis (left ear positive) and the z is the vertical axis (dorsal positive) (see Fig 1 for these conventions).

### Dissection and measurement

Eleven adult guinea pigs with weights from 311 to 487 g (mean 381 g) were anaesthetized with barbiturate overdose and the tissue removed from their skulls. Dental cement was applied to the skull sutures to prevent dislo-



Fig. 1. An overview of a guinea pig skull from a right vent posterior to the interaural axis to show the structure of Fig. 3 and Fig. 4 A, B, C.

skulls were dried and for each animal the osseous canal of every semicircular canal was exposed along as much of its length as possible by drilling. An attempt was made to standardize this dissection, so that for all animals the lateral-most bone in each canal was removed (see Figs. 2 and 3). The bony external auditory meatus and most of the bullae were left intact, permitting the skull to be mounted in a Kopf guinea pig stereotaxic head frame (Model 1216) in fairly normal fashion. Standard guinea pig ear-bars were used and the height of the incisor bar was set at 0. Thus the positioning of the skull in the stereotaxic apparatus was the same as that which would be used with a living guinea pig.

Kopf electrode carriage with micro-manipulator controls (model 1260) was placed in the stereotaxic frame and its planes of motion were aligned with the stereotaxic axes. About 50 mm of fine (80  $\mu$ m diameter) glass steel wire insulated except at the tip was affixed to the electrode holder and connected to a voltage follower, audio amplifier and loudspeaker. Touching the tip of this wire

to an osseous canal or reference point resulted in a loud "pop" which considerably assisted in identifying the "touch point". The reference point consisted of a short piece of 80  $\mu$ m wire cemented to the top of a bolt. The cement was filed so the tip of the wire was flush with the surface. This reference point was bolted to the stereotaxic frame and it remained untouched during all measurements.

Each skull was placed in the stereotaxic device and its position was not changed during measurement. The  $x$ ,  $y$  and  $z$  coordinates of a series of approximately equally spaced points along each canal were obtained by advancing the wire probe until it just touched the medial most wall of the osseous canal and reading the scales to the nearest 0.05 mm. Points in the ampullary and common crus regions were avoided. An average of 28 points per canal were recorded (range 17 to 50). After measuring the canals in one labyrinth a check was made to ensure the probe had not been displaced and if this check was satisfactory the probe was repositioned to enable readings of the contralateral labyrinth. The coordinates of



Fig. 3. An enlargement of Fig. 1. It shows the osseous semicircular canals are shown schematically in Fig. 4 A, B, C.

points along a semicircular canal were always referred to the reference point which was in turn referred to the stereotaxic zero (the point of intersection of the three planes defined above) thus relating all canal measurements to stereotaxic zero.

## RESULTS

### Statistical treatment

For each skull the raw data consisted of the  $x$ ,  $y$  and  $z$  coordinates of points along each canal. This data was punched onto cards and the coordinates translated into stereotaxic space

by subtraction. This data was plotted on a printer to ensure there were no punch errors, outlying data points or substantial gaps between data points along any canal. No error occurred for the anterior canals of any animals because of breakage during dissection of the extremely thin part of the anterior canal near the common crus (see Fig. 2). Three animals were excluded from all further analysis. One animal was excluded entirely because a detailed analysis showed it had not been positioned properly in the stereotaxic box. The plane was fitted to the data for each canal by the least squares technique and by principal

*Equations of the planes parallel to the circular canals passing through the stereotaxic zero*

Figure in brackets below each coefficient is the standard deviation

Horizontal	+ 716x (± 008)	- 263y (± 070)	- 637z (± 011)	=0
Anterior	- 578x (± 013)	+ 793y (± 008)	- 167z (± 018)	=0
Posterior	- 771x (± 011)	- 380y (± 014)	- 503z (± 010)	=0
Horizontal	+ 716x (± 008)	+ 263y (± 020)	- 637z (± 011)	=0
Anterior	+ 578x (± 013)	+ 793y (± 008)	+ 167z (± 018)	=0
Posterior	- 771x (± 011)	+ 380y (± 014)	- 503z (± 010)	=0

**II analysis** As in earlier studies both techniques gave virtually identical results but the results of the principal component analysis are reported here. This technique gives the direction cosines of a plane passing through the origin parallel to the plane of best fit through the data points of a given canal (Thursfield, 1957). In other words the *D* term of the normalized equation of a plane in three dimensions

$Ax + By + Cz + D = 0$  has been set to zero (Bers, 1969). The equations of the plane parallel to each canal are presented in Table I. The coefficients in these equations are the mean direction cosines. Since magnitude differences between direction cosines from left and right labyrinths were small, they were combined to improve estimation. This average value was then given the correct sign for the appropriate labyrinth.

For each animal the direction cosines of its canal planes served as input for another program which computed the angles among the canal planes and between each canal plane and the stereotaxic planes. Magnitude differences for angles from left and right labyrinths were small and hence these angles were combined wherever possible to improve estimation. These mean angles are presented in Table II together with the numbers upon which each mean is based, the standard deviations and two-tailed 95% confidence intervals (Guenther, 1965). Figs 2 and 3 show the orientation of the canals in a guinea pig skull and provide the perspective for Fig 4 A, B, and C.

**II Means, number of measurements (n), standard deviations and 95% confidence limits for angles in degrees, among ipsilateral canal planes, between contralateral synergistic canal planes and between each canal plane and the stereotaxic planes. The locations of these last set of angles are shown in Fig 4 (see Column 1).**

Location	Angle between the plane of the		Mean	n	S D	95% confidence limits	
						Lower	Upper
A	Horizontal canal	Anterior canal	122.15	17	6.13	118.99	125.30
	Anterior canal	Posterior canal	76.71	17	5.49	73.89	79.53
	Horizontal canal	Posterior canal	82.36	20	4.74	80.11	84.58
B	Left horizontal canal	Right horizontal canal	30.82	10	10.05	23.63	38.01
	Left anterior canal	Right posterior canal	37.17	8	4.42	28.47	35.87
	Left posterior canal	Right anterior canal	36.16	9	4.86	30.81	41.51
A θ <sub>1</sub>	Horizontal canal	Stereotaxic frontal	44.17	70	2.98	42.78	45.57
A θ <sub>2</sub>	Horizontal canal	Stereotaxic horizontal	50.31	20	3.80	48.53	52.09
A θ <sub>3</sub>	Horizontal canal	Stereotaxic sagittal	105.34	20	5.43	102.80	107.88
B θ <sub>1</sub>	Anterior canal	Stereotaxic frontal	54.71	17	3.82	52.75	56.67
B θ <sub>2</sub>	Anterior canal	Stereotaxic horizontal	99.78	17	4.36	97.54	102.02
B θ <sub>3</sub>	Anterior canal	Stereotaxic sagittal	37.36	17	3.24	35.69	39.03
C θ <sub>1</sub>	Posterior canal	Stereotaxic frontal	39.34	70	4.39	37.29	41.40
C θ <sub>2</sub>	Posterior canal	Stereotaxic horizontal	59.77	20	2.84	58.44	61.10
C θ <sub>3</sub>	Posterior canal	Stereotaxic sagittal	67.60	20	3.99	65.74	69.47

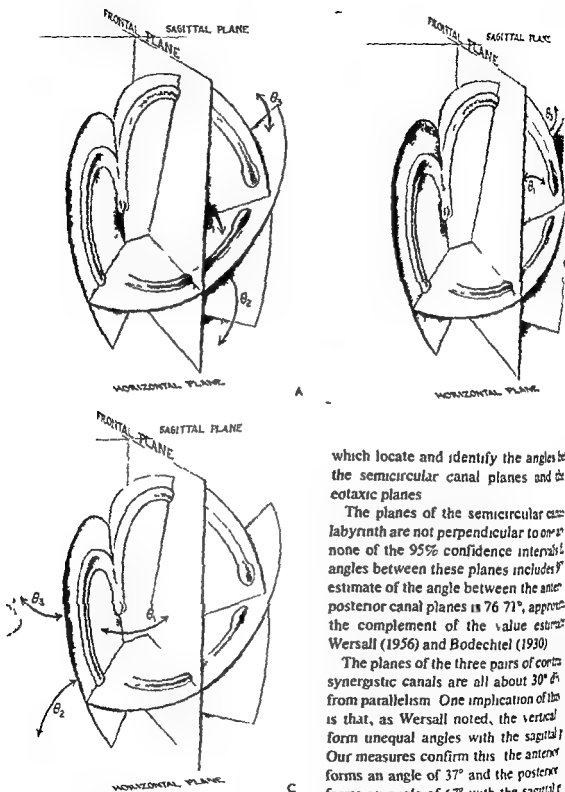


Fig 4 A B C The intersection of the semicircular canal planes on the right side of the head with the guinea pig stereotaxic planes.  $\theta_1$ ,  $\theta_2$ ,  $\theta_3$  identify the angles between the canal plane and each stereotaxic plane; the magnitudes of which are given in Table II

which locate and identify the angles between the semicircular canal planes and the stereotaxic planes

The planes of the semicircular canals in the labyrinth are not perpendicular to one another. None of the 95% confidence interval estimates of the angles between these planes includes 90°. Our estimate of the angle between the anterior-posterior canal planes is 76.71°, approximately the complement of the value estimated by Wersall (1956) and Bodechtel (1930).

The planes of the three pairs of corticostriate synergistic canals are all about 30° from parallelism. One implication of this is that, as Wersall noted, the vertical canals form unequal angles with the sagittal plane. Our measures confirm this: the anterior canal forms an angle of 37° and the posterior canal forms an angle of 67° with the sagittal plane.

Each horizontal canal forms an angle of 50.31° (open anteriorly) with the horizontal stereotaxic plane and is inclined (lower by 105.34°) from the stereotaxic sagittal plane. The vertical canals are not

### III Magnitude of pitch and roll manoeuvres in degrees and direction of rotation needed to bring any semicircular canal into the horizontal plane of rotation

pitch nose down PTD—pitch tail down PRED  
right ear down RLED=roll left ear down

	Pitch	Direction	Roll	Direction
horizontal	48.37	PND	15.34	RRED
anterior	73.48	PTD	52.64	RLED
posterior	56.83	PTD	22.40	RRED
horizontal	48.37	PND	15.34	RLED
anterior	73.48	PTD	52.64	RRED
posterior	56.83	PTD	22.40	RLED

Similar to the stereotaxic horizontal plane the anterior canal forms an angle of 99.78° with the posterior canal an angle of 59.77° with the horizontal plane. This nonperpendicularity would have resulted in errors if the plane angles had been estimated by Anderson & Valentiniuzzi's (1968) projection method.

For the purpose of positioning a guinea pig for angular acceleration stimulation the pitch and roll angles of each canal are needed. The pitch angle is that angle formed between the plane of intersection of the canal plane and the horizontal plane measured along the nasooccipital axis. After pitching the animal by the required amount the roll angle is the angle formed between the interaural axis and the line of intersection between an earth vertical plane and the horizontal plane and the horizontal plane. The pitch and roll angles for each canal and the direction of rotation for physiological stimulation by an earth horizontal plane of rotation are given in Table III. In order to position the animal the rotations are not independent (Goldstein, 1965) and the order of the manoeuvres must be pitch then roll. The manoeuvres start from the skull being in the standard stereotaxic position shown in Figure 1. So for example to bring the right anterior canal into an (earth horizontal) plane of rotation requires pitching the head 73.48° nose down and then rolling the animal 52.64° right down.

### Translation to other stereotaxic systems

Luparello (1967) specified that when the heights of the two points on the skull surface 6 mm and 14 mm anterior to the interaural axis were identical the guinea pig skull was horizontal. In our stereotaxic system these points were an average of 1.10 mm discrepant ( $\pm 0.50$  standard deviation). These points would be at the same height if the skull were pitched nose down by 7.38°. Thus to translate the positioning angles presented above into Luparello's system requires a pitch nose down of 7.38°; the pitch angle of the horizontal canals becomes 40.54° open anterior.

The inferior margins of the medial canthi were on average 21.79° above ( $\pm 1.35$ ) the stereotaxic horizontal plane. Translation to Tindal's stereotaxic system requires a pitch nose down by this amount; the pitch angle of the horizontal canals becomes 26.58° open anterior.

In some definitions of stereotaxic horizontal for rodents the incisor bar is set 2.5 mm below the external auditory meatus (Hurt et al., 1971). In our skulls this corresponds to a pitch of 3.34° ( $\pm 0.12$ ) nose down.

The junction of the coronal suture and the superior orbital ridge was 40.13° above ( $\pm 1.51$ ) the stereotaxic horizontal plane. This point was measured as being the most probable to lie on Kristensen's line between the external meatus and the superior margin of the orbit. As an estimator of the pitch of the horizontal canal Kristensen's line probably underestimates the true pitch by about 8° (48.37°–40.13°=8.24°).

### DISCUSSION

It is important to emphasize that the planes of the semicircular canals given in this paper are planes derived from measurement of the osseous semicircular canals. The extent to which these planes correspond to the planes of the membranous ducts depends on the width of the perilymphatic space. A. A. Gray (1907) and O. Gray (1951) have noted that the

perilymphatic space of rodents is usually very small or absent. This fact can be verified by a photograph of a decalcified guinea pig labyrinth in O. Gray (1955) and we have also confirmed it by measurements of guinea pig membranous labyrinths fixed in 10% formalin. Since the radius of curvature of the guinea pig canals is of the order of 1.90 mm (Jones & Spells, 1963; Curthoys et al., 1975a) and the perilymphatic space is of the order of 0.02 mm, it follows that any deviation between the plane of the osseous canal and that of the membranous duct would be about  $1^\circ$  ( $\arctan(0.02/1.90)$ ).

It should also be stressed that the statistical techniques we used provide a best fitting plane to the series of data points for a canal. It was apparent from inspection of the dissected osseous semicircular canals that there are systematic departures from a plane, particularly for the anterior and posterior canals. Nevertheless these departures are not large and for the purposes of this investigation it was deemed adequate to use a linear approximation. This issue of the planarity of semicircular canals will be considered in more detail in a forthcoming paper (Curthoys et al., 1975a).

One of the major aims of this study was to establish the optimal positioning of the guinea pig skull for physiological stimulation of a given set of semicircular canals and to determine the extent to which other canals would be stimulated when the head was in this optimal position. It is clear from Tables II and III that it is impossible to position any two canals to be parallel to a single plane of stimulation. The optimal position for bilateral stimulation of the horizontal canals is with the skull tipped  $48^\circ$  nose down relative to an earth horizontal plane of stimulation. In this position each horizontal canal is approximately  $15^\circ$  out of the plane of stimulation, the anterior canals both form an angle of  $71.19^\circ$  and the posterior canals both form an angle of  $75.91^\circ$  with the plane of stimulation. Thus when the guinea pig head is optimally positioned for stimulation of

both horizontal canals the vertical canals receive an appreciable proportion of stimulating angular acceleration. For example, if a guinea pig were positioned for stimulation of both horizontal canals and an angular acceleration of  $4^\circ/\text{sec}^2$  the anterior canals would both receive an acceleration of  $1.29^\circ/\text{sec}^2$  ( $\cos 71.19^\circ$ ), the posterior canals an angular acceleration of  $1.97^\circ/\text{sec}^2$  ( $\cos 75.91^\circ \times 4$ ). These levels of the vertical canals would be expected to influence the firing of sensitive primary and secondary vertical canal neurons (Curthoys, 1973) and could lead to misinterpretation in studies of convergence (Curthoys et al., 1971; 1973; Markham & Curthoys, 1972).

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### ZUSAMMENFASSUNG

Bei 10 ausgewachsenen Meerschweinchen wurden stereotaktische Koordinaten von einer Reihe von an jedem knöchernen Bodengange endigenden Kanälen um eine mathematische Gleichung für den Bodengang im stereotaktischen Raum zu erhalten. Die Beziehungen innerhalb der Gehörgänge und den Gehörgängen werden angegeben sowie die Orientierungen des Kopfes für den physiologischen Stimulus. Die Gangs oder jedes Paares von synergistischen Kanälen auf Ebenen der Bodengänge in jedem Labyrinth sind senkrecht zueinander und die Ebenen der konjugierten synergistischen Gänge weichen von der Parallelität ab.

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## INFLUENCE OF THE HYPOTHALAMIC STIMULATION ON VESTIBULAR NUCLEI UNITS IN THE RAT

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**Abstract** Electrical stimulation of rat hypothalamus influenced the activity of the vestibular nuclei units depending on the stimulus location. Vestibular units were excited predominantly by antero-lateral hypothalamic

In an earlier paper (Kubo et al. 1974) it was reported that the electrical stimulation of the vestibular nuclei induced the electrical responses in the hypothalamus bilaterally. The present paper is intended to demonstrate a descending influence from the hypothalamus to the vestibular nuclei.

It is generally considered that many factors such as disturbances of the endocrine system, autonomic nervous system and psychiatric stress are closely related to the inducement of vertigo or dizziness (Wodak, 1959). However, it is not clear how or why these factors cause this complaint. It has earlier been clearly demonstrated that there is close interaction between the peripheral autonomic system and vestibular system (Watanabe, 1967). At present, the role of the central autonomic nervous system in vertigo and/or dizziness, especially where the hypothalamus is concerned, is still obscure. Clinical experience suggests that the hypothalamus does play a part in the incidence and development of this disorder (Spencer, Harrison & Naftalin, 1968; Wood, 1968; Egami, 1972; Naito, 1973; Hinoki et al., 1973). It therefore seems very necessary to clarify the role of the hypothalamus in vertigo.

Prompted by the above mentioned considerations, the correlation between hypothalamus and vestibular nuclei has been investigated.

### MATERIALS AND METHODS

#### *Vestibular units response*

The experiments were conducted on albino rats. Surgical and recording methods were identical with those reported in the previous paper (Kubo et al., 1974). The rat was placed in a stereotaxic frame on the operation table, which moves sinusoidally with a period of 3-10 sec and total amplitude of 10°. The head was inclined downwards as much as possible due to the horizontal location of the lateral semicircular canal and fixed at the angle of rotation.

For the electrical stimulation, two stainless steel concentric bipolar electrodes with diameters of 0.2 mm and intertip distance of 0.5 mm were inserted through a small trephine and an intact cortex to the hypothalamus with the aid of the stereotaxic atlas (Pellegrino & Cushman, 1967). The electrode was aimed at the lateral hypothalamus (level head co-ordinates 0.5-1.0 mm behind bregma, 1.5 mm lateral to sagittal

### Effects of hypothalamic stimulation on vestibular neurons

	I	II	III	Total
to	24	33	1	58
nse				
ic	17	38	0	55
ion				
to	31	28	2	61
	14	17	■	31
	17	11	2	30

nd 7-9 mm beneath dura) and medial  
alamus (2-3 mm behind, 0.5 mm lateral  
9 mm depth)

recording the vestibular unitary re-  
sponse, the glass microelectrode, with a tip  
radius of 1-5  $\mu$ m and filled with lithium  
chloride, was inserted stereotactically through  
the occipital trephine and intact cerebellum  
to the vestibular nuclei. When a unit was  
identified, its response to rotation was

effect of hypothalamic stimulation on  
those identifiable neurons which re-  
sponded to sinusoidal rotational stimulus, was  
studied. An electrical stimulus was applied  
directly through the posteromedial and  
lateral electrodes in the hypothalamus.

Angular pulses were delivered with the  
following parameters: 30/sec, 0.2 msec, 5-10  
volts, maintained for 5-10 seconds.

#### Delayed evoked field potential

In a series of the above mentioned experi-  
ments, an attempt was made to identify the  
evoked field potentials within the vestibular  
nucleus by stimulation of the hypothalamus.  
In this case, single pulse stimulus was used  
(0.2-0.5 msec, 2-3/sec).

The evoked potentials were picked up  
polarographically between the glass micro-  
electrode and the reference electrode in the  
nucleus and were multiplied 30 times by the

computer and photographed with a polaroid  
camera.

The sites of the hypothalamic stimulation  
were determined by making an electrolytic le-  
sion at the tip of the electrode. The recording  
electrode tip position was established by the  
development of red spots using the technique  
of Mitarai (1960).

## RESULTS

### Response to angular stimuli

Fifty eight vestibular neurons were identified  
and classified by their response to sinusoidal  
rotational movement (Shimazu & Precht,  
1965). Twenty four were type I, 33 type II,  
and 1 type III (Table I).

Fig 1 shows the original action potentials  
from a single cell located in the left medial  
vestibular nucleus during sinusoidal rotation at  
a frequency of 0.2 Hz. The upper illustrated  
curve indicates the position of the turntable  
(up-left going).

### Response to hypothalamic stimulation

The hypothalamus was systematically stimu-  
lated in all of the hypothalamic areas which are  
medial to the fornix and extend from the  
rostral portion of the mammillary body in the  
infundibular region of the ventromedial hypo-  
thalamic nucleus and lateral to the fornix and  
from the rostral portion of the infundibular  
region to the chiasma optica.

Two areas in particular responded to hypo-  
thalamic stimulation. These were the anterior  
hypothalamus in the lateral hypothalamic area  
of the infundibular region (AH), and the mid-  
dle hypothalamus in the medial hypothalamic  
area of the same region (MH) which is almost  
at the level of the ventromedial nucleus. In all  
examined animals it was histologically ver-  
ified that the anterolateral electrode track was  
located in the AH and the posteromedial  
electrode track was positioned in the MH.

Fig 2 demonstrates the facilitatory response

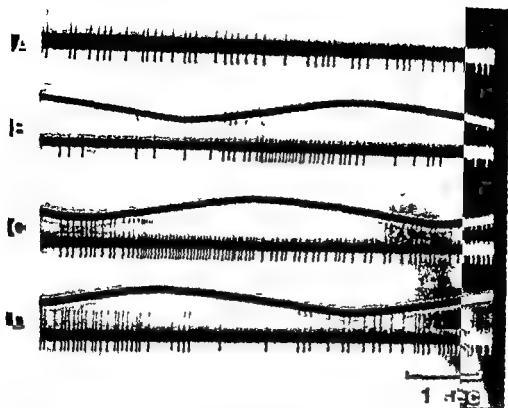


Fig 1 Original records of sinusoidal rotation (period 5 sec total amplitude  $110^\circ$ ) and original AP train A indicates the spontaneous discharge in Type I neuron in the

left vestibular nuclei In B C D the upward angular displacement indicates leftward movement

of the spontaneous discharge in a vestibular neuron to the electrical stimulation of the AH

Fig 3 shows the inhibitory response of the spontaneous discharge in another vestibular neuron to the stimulation of the MH

Each vestibular neuron was subjected to two kinds of stimulation, AH and MH, so that while 58 neurons were examined in this experiment, the responses elicited totalled twice this figure, i.e. 116. Thus, of the 58 neurons included in the analysis, 61 positive responses were evoked by hypothalamic stimulation, leaving a negative response total of 55. Of the 61 positive responses, 31 responded with facilitation, and the remaining 30 responded with inhibition. Thus, about half the neurons responding to sinusoidal rotation responded to hypothalamic stimulation with a corresponding degree of facilitation or inhibition.

Of 24 type I neurons 65% (31 positive re-

sponses out of 48) responded to hypothalamic stimulation. In contrast, in 33 type II, the positive response totalled 44% (23 responses out of 66) (Table 1).

It seems that the type I neuron is more strongly influenced by hypothalamic stimulation than the type II neuron. On the other hand, there was no significant difference in the proportion of positive responses evoked by hypothalamic stimulation with either rotation or facilitation in the group of neurons, as compared with the group of neurons.

#### Summated evoked field potential

Prompted by the above mentioned findings, the responses of the vestibular activity to electrical stimulation of AH and MH were examined. Some differentiation in the evoked field potentials within the

10 Effects of stimulation of the anterior hypothalamus  
 15 lateral hypothalamic area (AH) upon spontaneous  
 17 discharge of the ipsilateral vestibular neuron (Type II) A  
 20 spontaneous discharge B onset of stimulation C during

stimulation D immediately after end of stimulation The  
 short downward line indicates the artifact of stimulation (6  
 V 0.5 msec 30 Hz) E temporary inhibition after stimula-  
 tion

ir nuclei by a single pulse stimulus of the  
 thalamus. All neurons which responded  
 sinusoidal rotatory movement failed to re-  
 spond to a single pulse stimulus of the hypo-  
 thalamus. However, we were able to record  
 a summated evoked field potential in the ves-  
 tibular nuclei in MH as well as in AH (Fig. 4).  
 In other words the electrical stimulation in  
 AH produced the evoked potential with a  
 monophasic negative wave of about 15–20  
 msec peak latency.

While the electrical stimulation in AH also  
 produced a monophasic negative evoked po-  
 tential of the same latency, it sometimes had a

tendency to elicit a monophasic positive wave  
 (Fig. 4).

The phase reversal (turn over point) of the  
 summated evoked potential was not recorded  
 and the amplitude was also of the same order  
 in cerebellum and reticular formation. Follow-  
 ing these results the relation between the de-  
 velopment of the evoked potential in the ves-  
 tibular nuclei and the response of spontane-  
 ously active vestibular neurons by the electri-  
 cal stimulation of AH and MH was examined.  
 Table II sets out the summarized results. In  
 positive cases of the summated evoked poten-  
 tial 10 of the 27 neurons in AH stimulation

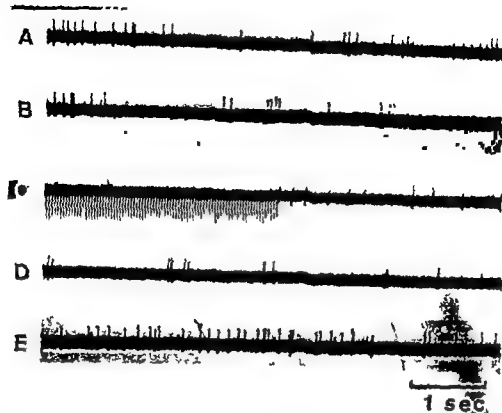


Fig. 3 Effects of stimulation of the middle hypothalamus in the medial hypothalamic area (MH) upon spontaneous discharge of the other ipsilateral vestibular neuron (type II). *A* spontaneous discharge *B* onset of stimulation *C*

immediately after end of stimulation. The down indicates the artifact of stimulation (6  $\times$  0.2 sec). *D* temporary inhibition after stimulation *E* facilitation.

responded with a facilitation of spontaneous activity, 6 with inhibition and 11 with no response. In cases of the non-evoked potential, 6 of the remaining 31 exhibited facilitation, 7 inhibition and 18 no change of spontaneous discharge in AH stimulation.

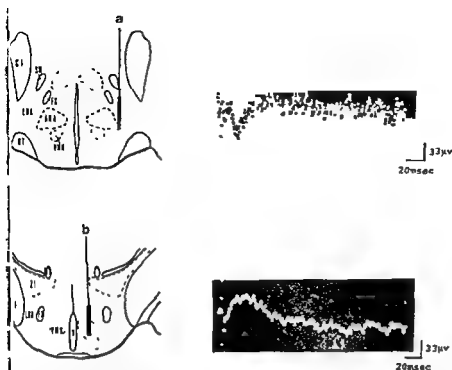
In MH stimulation, 6 of the 28 neurons in the presence of the evoked potential responded with a facilitation of spontaneous activity, 12 with inhibition and 10 with no response. However, of the remaining 30 neurons in cases of non-evoked potential, 9 exhibited facilitation, 5 inhibition and 16 no change of spontaneous discharge in MH stimulation. Thus, the AH stimulation is apt to facilitate more easily the spontaneous discharge in the vestibular neurons, and the MH stimulation seems to inhibit more easily the spontaneous discharge, when the evoked response is recorded in AH and MH stimulation.

## DISCUSSION

Generally speaking, there seem to be two schools of thought concerning the function of the autonomic nervous system in the thalamus.

One is that the lateral hypothalamus belongs to the parasympathetic division and that the medial hypothalamic area belongs to the sympathetic division. Yuasa (1964) reported that the electrical stimulation of the lateral hypothalamic area increased the spontaneous discharge and also that the stimulation of the medial hypothalamus increased the cervical sympathetic discharge in rabbits.

On the other hand, according to Göttsche (1967) the anterior hypothalamic area is related to the parasympathetic effects and the posterior hypothalamic area to the sympathetic effects.



Schematic drawing of the hypothalamus and the stimulating sites (left column) and summated evoked field potentials in vestibular nucleus (right column). Left column indicates electrode track positioned at the anterior hypothalamus in lateral hypothalamic area (AH) and b indicates electrode track positioned at the middle hypothalamus in medial hypothalamic area (MH). Right column upper picture: positive evoked potential from the

stimulation of AH (3 V 0.5 msec 3 Hz) average of 30 cycles. Lower picture: negative evoked potential from the stimulation of MH (3 V 0.5 msec 3 Hz). Abbreviations: AHA: anterior hypothalamic area; CI: capsula interna; EX: fornix; LHA: lateral hypothalamic area; OT: optic tract; SI: stria medullaris thalami; V: ventriculus; VMH: ventromedial nucleus of hypothalamus; ZI: zona incerta.

Results of our experiments have demonstrated that the activity of vestibular neurons responding to the sinusoidal rotatory movement was altered in two ways, facilitation and inhibition, by the electrical stimulation of certain areas of the hypothalamus. Furthermore, two sorts of summated evoked potentials from the vestibular nuclei, monophasic negative and positive, were elicited by the repeated pulse stimuli of some areas of the hypothalamus. Interestingly, in AH stimulation the activity of vestibular neurons responded predominantly with a facilitation in five cases of monophasic-negative and positive evoked potential, and in MH stimulation it responded mainly with an inhibition in five cases of monophasic positive evoked potential. Concerning the descending influ-

ence from the forebrain to the vestibular neurons, Arslan & Molinari (1965) reported that the vestibular neuronal activity was modulated in terms of facilitation or inhibition by the electrical stimulation in certain areas of the cortex. According to Gildenberg & Hassler (1971) most vestibular neurons were facilitated by the cortical stimulation.

It has been shown by Cooper & Rolls (1974) that units in the pons and medulla are activated by the self-stimulation of the lateral hypothalamic region and sites along the medial forebrain bundle in the rat.

From anatomical data regarding hypothalamic fiber connection it is well known that the descending efferent pathways of the hypothalamus are contained mainly in the dorsal longitudinal fasciculus and the medial

Table II

( ) positive wave

	Facilit	Inhibit	No response	Total
<i>Hypothalamic stimulation (ant hypothalamus)</i>				
Evoked response (+)	10 (3)	6 (2)	11 (3)	27 (8)
Evoked response (-)	6	7	11	31
Total	16 (3)	13 (2)	29 (3)	58 (8)
<i>Hypothalamic stimulation (mid hypothalamus)</i>				
Evoked response (+)	6	12	10	28
Evoked response (-)	9	5	16	30
Total	15	17	26	58

forebrain bundle (Nauta & Haymaker 1969) Ban (1964, 1966) reported that there is a polysynaptic neural circuit between the medial hypothalamic area and the vestibular nuclei via the dorsal longitudinal fasciculus, as we have verified electrophysiologically (Kubo et al., 1974). Thomas & Calaresu (1974) stated that the electrical stimulation of the posteromedial hypothalamus inhibited the vagal bradycardia elicited by stimulation of localized medullary region and that it was probably mediated by the dorsal longitudinal fasciculus from the existence of the two sorts of biphasic field potentials.

Therefore, the difference in the response of vestibular neurons and in the polarity of the summated evoked potential to the hypothalamic stimulation appears to depend on the difference in the stimulation site of the hypothalamus and in the descending neural circuit from the hypothalamus to the pons and medulla including the vestibular nuclei.

We confidently suggest that the electrical stimulation of the central sympathetic division inhibits the spontaneous discharge in the vestibular neurons and the same stimulation of the central parasympathetic division facilitates the spontaneous discharge.

Although we do not completely deny the electrical spread in such a stimulation of the hypothalamus there seems to be no doubt

that there are differing responses to the stimulation of such a circumscribed part of the hypothalamus, as recent evidence comes to light that a set of neurons which, when activated, elicit an autonomic functional response in this area (Oertel 1964).

It is therefore quite probable that the tubular neuronal activity will be modulated by the hypothalamic descending influence. This produces two different effects being sympathetic or parasympathetic through the medial forebrain bundle and the dorsal longitudinal fasciculus.

However, the question arises as to whether the modulation of the vestibular activity is induced only by the aforementioned effect from the hypothalamus to the vestibular nuclei, as it has been known that hypothalamic stimulation induced the secretions of noradrenalin and adrenaline in varying proportions depending on the location of the stimulus in cats (Folkow & 1954). This excreted noradrenalin and adrenaline may possibly modulate the vestibular neuron activity (Yamamoto 1967). Concerning this problem we shall make further studies and shall report our findings in the future.

It is considered likely that the disturbance of the hypothalamic function in other cases is more closely related to the disturbance of the autonomic nervous system, the disturbance of the endocrine system and psychiatric diseases.

The hypothalamic excitation is assumed to modify the level of the vestibular activity and influence the incidence and development of vertigo and/or dizziness.

#### ACKNOWLEDGEMENT

Gratitude is expressed to Assistant Professor Matano for helpful suggestions during this study.

#### ZUSAMMENFASSUNG

Die elektrische Reizung des Hypothalamus beeinflusst einige Einfluss auf die Tätigkeit der vestibulären Neurone.

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 ten wurden durch die Reizung im anterioren  
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## THE EFFECT OF GRAVITY ON POSITIONAL ALCOHOL NYSTAGMUS PHASE II IN MAN

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(Received April 12 1975)

**Abstract** On request from the Swedish state authorities for health and civil air transport (Socialstyrelsen and Luftfartsstyrelsen) experiments were performed in order to establish the duration of the vestibular disturbances after alcohol intake related to rules for aviation safety. It is known that the threshold for provocation of positional alcohol nystagmus (PAN) is lowered under the influence of gravity forces above normal. It has been suggested that PAN II could be provoked by  $g$  loads as late as 48 hours after a small alcohol intake. Our experiments were performed in a human centrifuge with  $g$  values 2 and 3. Whisky (43 vol% 2.5 cc/kg b.w.) was given in a single dose and blood alcohol analyses were frequently taken. The results show that vestibular disturbances appearing as  $g$  provoked PAN II do not persist for any considerable time. Consequently the present limit of 24 hours' sobriety before flight seems sufficient. Positional nystagmus provoked by  $g$  load even without alcohol intake in the test persons is a new observation.

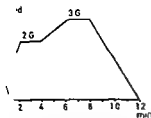
As early as 1842 Fluorens described nystagmus as one of the consequences of alcohol intoxication. Barany (1911) and Barany & Rothfeld (1913) pointed out that this alcohol induced nystagmus was dependent upon the position of the subject's head when examined. In a series of investigations Aschan et al. (1955, 1956, 1958 and 1961) in controlled human experiments using ENG and simultaneously monitoring the blood alcohol curve showed that after a single dose of alcohol positional alcohol nystagmus (PAN) could be recorded and quantitatively

analysed. For details the reader is referred to the references given above. It is essential for the purposes of the present investigation that PAN II should persist as late as 14 h after the last alcohol intake. An interval of 24 h between the last alcohol ingestion and the start of activity should, according to the present experiments, guarantee that the vestibular consequences of alcohol intoxication are excluded and the pilot fit for flying duties.

Bergstedt (1961) showed that  $g$  loads of 1-3  $g$  increased the intensity of PAN. In a few experiments even PAN II in the mentioned experiments were few and far between. If performed relatively soon after the last alcohol intake when alcohol still was present in the blood Bergstedt also showed in some experiments that positional nystagmus other than PAN II often intensified when the  $g$  load increased. In healthy normal test persons without alcohol  $g$  loads did not provoke nystagmus in Bergstedt's experiments. He used a human centrifuge so arranged that angular acceleration was a source of error which was excluded.

Oosterweld et al. (1969, 1969 and 1970) have shown that PAN I and PAN II are abolished in weightlessness as reached in parabolic flight. They also investigated a few test persons during the period after PAN II increased by flying in tight circles with  $g$  value to 3. They report nystagmus findings with  $g$  load resembling PAN II up to 4-5  $g$ .

The experiments were supported by Swedish Medical Council grant number B71-40X 3305 01G (1368/70 Aschan).



Relationship between angular speed resultant and time during every experiment

ng the alcohol intake. At present the during which alcohol drinking is forbid r pilots is 24 hours before flight, but the of Oosterweld et al would double this. Hence a thorough investigation was ted by Luftfartsstyrelsen and Social en.

## MATERIAL AND METHODS

een persons have been used in com sts: 12 men aged 20–36 years (mean age y body weight 54–91 kg (mean 74), 5 wom d 18–26 years (mean 21) body weight e (mean 60). The test persons were vol s from the Police School, the Medical

School and the University of Stockholm. The centrifuge was the one at the Aviation and Naval Medicine Institution, Karolinska Institutet, Stockholm, used by Bergstedt (1961) to whom the reader is referred for details. The test person had the force resultant from gravity and centrifugal force in his binocular line. The  $g$  values used were 2 and 3  $g$ . The angular acceleration never exceeded  $0.9^\circ/\text{sec}^2$  so as not to induce rotatory nystagmus. Supporting cushions were placed at the forehead and occiput to avoid coriolis forces. Usual ENG technique described by Aschan et al (1956) was used. The test leader was in constant contact with the test person by a two-way intercom and by a TV camera watching the test person's head and neck. Before every new registration, blood samples were taken to dis- close any (forbidden) alcohol consumption.

The test procedure was as follows:

### Before alcohol intoxication

1. An interview was made in order to exclude test persons with illnesses, especially related to hearing and balance medication, or suspected alcohol abuse. No alcohol intake

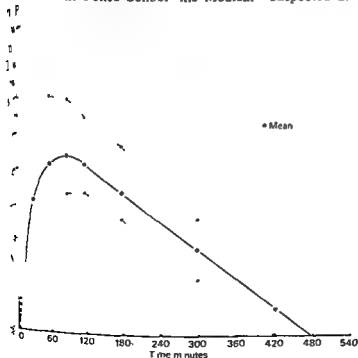


Fig. 2. Blood alcohol curves. The unbroken line shows the mean value for all the different test persons. The maximum and minimum alcohol curves are also drawn.

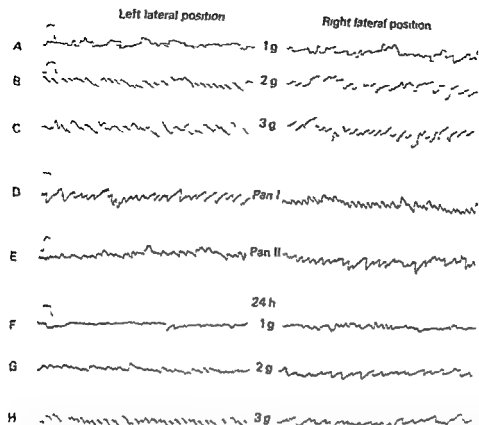


Fig. 3. Sequence of electro nystagmogram test person group 2. Right beating nystagmus in left lateral position and left beating nystagmus in right lateral position at 2 and 3 g (B and C) in the sober test person and no positional nystagmus at 1 g (A). D shows PAN I and E PAN II. After

24 hours the nystagmus at 2 and 3 g (F and G) with B and C. The calibration for 10° eye in each registration makes this quantitative possible. The blood alcohol concentration was 0 and at E 0.67 g/100.

was permitted for 48 hours preceding the experiment.

2. Electronystagmography (ENG) was performed with the test person lying back and in right and left lateral positions with closed eyes.

3. ENG-registrations without alcohol were made in the centrifuge under  $g$  values 2 and 3 with the test person in right and in left lateral position. The running schedule is presented in Fig. 1.

#### *Alcohol intoxication and later*

4. 2.5 cc whisky per kg body weight was ingested in 10 minutes. Blood alcohol test was taken before the alcohol administration and frequently thereafter (see Fig. 2).

5. Registration by ENG of PAN I and PAN II was performed.

6. Centrifuge experiments with 2 and 3 g were performed several times. PAN II could no longer be evoked. Right lateral positions were used according to schedule in Fig. 1.

Blood alcohol analyses were taken from each test person frequently enough to construct a blood alcohol curve for each subject (see Fig. 2). The analyses were performed by gas chromatography at the Institute of Clinical Chemistry, Regional and Local Hospital of Linköping. This method gave reliable and accurate results with very small

## RESULTS

The unintoxicated test persons were registered in right and left lateral position at 1, 2 and 3 g. Twenty different test persons

The test persons reacted in four different ways

*up 1* In six tests the subjects did not show any nystagmus at any of the  $g$  values 1-2

*up 2* Nine test persons were free from nystagmus at 1  $g$  but had nystagmus findings at 2  $g$ . This nystagmus was in all the eleven tests right beating nystagmus in left position and left beating nystagmus in lateral position which could have been interpreted as PAN II if pre alcohol tests had been performed

*up 3* Even at 1  $g$  2 test persons showed minimal nystagmus similar to that described for group 2. This nystagmus appeared at 2 and 3  $g$

Four test persons had to be excluded because at 1  $g$  they showed a spontaneous nystagmus uninfluenced by position. They tested un intoxicated at 2 and 3  $g$  but as 3 of them had a syncope during  $g$  loading they were all excluded

For each test person the time was noted when PAN under  $g$  load was last seen and when the test showed absence of PAN. For groups 2 and 3 absence of PAN is replaced by nystagmus comparable to the pre alcohol findings. The mean duration of freedom from alcohol ingestion during which PAN could be re evoked by  $g$  loading was *up 1* 18 hours and for group 2 19 hours. The last test finding of PAN under  $g$  load was 24 hours in one single test person but he belonged to group 3 and the pathological positional nystagmus in the un intoxicated state is a source of error

## DISCUSSION

PAN II is almost exclusively seen in man (1967). Ödkvist & Oosterweld in preparation. In their experiments had to be performed on 10 test subjects. Furthermore the experiments were performed in order to solve problems concerning aviation safety. In order to be able to obtain good recordings without unnec-

essary disturbances and risks for the test persons we used a centrifuge to produce the  $g$  values instead of the other expensive possibility—that is an airplane. The choice of centrifuge experiments instead of airplane experiments appeared to be successful. As seen in Fig. 1 the  $g$  loads could be so prolonged as to eliminate rotatory provoked nystagmus as a source of error. Slow accelerations from 1 to 2  $g$  and from 2 to 3  $g$  were chosen in order to avoid rotatory induced nystagmus.

The reaction pattern in group 2 has never been described before in a sober test person showing no positional nystagmus at 1  $g$  but obtaining positional nystagmus at 2 and 3  $g$ .

The possibility that the findings were different if the test person had not only the head but also the body in lateral position was investigated. This was done on the suspicion that with the head in lateral position but the body not totally turned nystagmus of a cervical origin might appear. No difference was seen however between these two variations of position in the centrifuge. The clean conditions in centrifuge experiments compared with airplane experiments do not even in this connection produce as great risks for sources of error.

The greatest sources of error are excluded by using in every experiment the same centrifuge, the same position and the same schedules for the alcohol intake and the  $g$  loads. In order to avoid habituation the test procedure for every volunteer could not be repeated too often. It should be observed that the nystagmus findings in groups 2 and 3 under  $g$  load were always of the right beating type in left lateral position and left beating nystagmus in right lateral position. These nystagmus directions are the same as is shown by positional alcohol nystagmus phase II (PAN II) without the 0-experiments. These test persons some hours after the alcohol intake could have been mistaken as still having PAN II during a load of higher  $g$  values. The doses of alcohol were systematically related to body weight in order to yield the same level of intoxication in the

different test persons. The dose was chosen to give a maximum blood alcohol level punishable for car drivers according to Swedish law. The dose chosen was based on some thousand earlier human controlled alcohol experiments (Aschan et al., 1956, 1957, 1958, 1961 and 1964). The test persons were instructed not to touch alcoholic beverages for 48 hours before the procedure but the test showing no blood alcohol before the experiment was the only way to exclude earlier PAN II as a source of error. Fig. 2 shows that the amount of alcohol administered resulted in a blood alcohol concentration that according to Swedish law for car drivers is criminal. The figure also shows that although the given amount of alcohol per kg bodyweight was the same, and ingested into an empty stomach, the spread in blood alcohol concentration among the test persons was considerable.

The conclusion of the experiments is that PAN II can be re-evoked by higher  $g$  values. No proof has been found, however, that PAN II can be evoked after a longer period of time—24 hours between alcohol intake and flight seems to be enough. This is valid for an alcohol intake not exceeding 2.5 cc alcohol per kg body weight i.e. 20 cl whisky for a man of 80 kg. The finding that 9 sober test persons out of 17 not having any positional nystagmus when  $g$  loaded showed findings identical to PAN II, was a new and surprising observation. This might be a source of error in similar experiments if not considered.

### ACKNOWLEDGEMENTS

The author acknowledges Prof. Hilding Bjurstedt supplying personnel and equipment at Inst. of Aviation Medicine, Karolinska Institutet, Stockholm. I wish to thank Prof. Gunnar Aschan for advice and Laboratory Technician Håkan Barrang for technical assistance.

### ZUSAMMENFASSUNG

Experimente zur Dauer vestibulärer Störungen nach Alkoholkonsum wurden durchgeführt um brauchbare Daten für die Flugsicherheit zu gewinnen. Es ist bekannt dass die Provokationsschwelle des Alkohollagennystagmus

bei  $g$ -Belastungen gesenkt wird. Man hat früher publiziert die Alkohollagennystagmus durch erhöhte Schwerkraft während 48 Stunden nach Alkoholkonsum gezeigt haben. Wir geben Whisky (43 vol% 2.5 cc/kg) und bestimmen Alkoholspiegel häufig. Unsere Experimente zeigten, dass die Alkohollagennystagmus dauert nicht länger als die geforderte Sicherheitszeit für Piloten von 24 Stunden. Ein bedeutungsvoller neuer Befund war dass 9 Personen ohne Alkoholeinfluss unter  $g$ -Belastung Lagenystagmus zeigten.

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 effect of air pressure and oxygenation on the duration  
 of positional alcohol nystagmus

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s of the organ of Corti were dissected the temporal bones, stained by a modified (see *infra*) of the silver pyridine method of Holmes (McManus Mowry, 1960) studied as whole mount or surface preparations with the light microscope. The experimental material was compared with similarly prepared preparations from normal animals. In experimental lesions were made in the dorsal aspect of the brain stem by the method described in our previous study (Wright & Eaton 1973). Successful lesions were performed in 14 animals. The post-operative survival times ranged from two days to four weeks; however most animals were allowed to survive from one to three weeks. The exact survival times are given in the following table.

Post-operative survival time (days)	Number of animals
1	1
2	1
3	3
4	4
5	1
6	3
7	1

In each case the extent of the lesion was fully determined by a study of brain stem sections prepared according to the method of & Eichman (1959). Fig. 1 illustrates diagrammatically the positions of the lesions. In 11 animals the lesion extended inward from the surface of the brain stem just lateral to the sulci limitans to an area just inside the spinal tract of the fifth cranial nerve (position indicated by the solid wedge). In four cases the lesion had a more medial position and included the descending root of the facial nerve as indicated by the broken wedge. In all animals the lesion extended approximately one millimeter rostral and caudal to the level of the genu of the facial nerve. The cochleas on the side contralateral to the lesion were processed in parallel with those on the operated side. Additional control

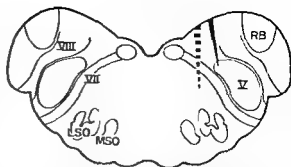


Fig. 1. Diagrammatic cross section of guinea pig brain stem at the level of the facial genu. The solid and broken wedges (upper right) indicate the positions of lesions discussed in the text. VIII, vestibulo-cochlear nerve; VII, facial nerve; V, nucleus and spinal tract of trigeminal nerve; LSO, lateral superior olivary nucleus; MSO, medial superior olivary nucleus; RB, restiform body.

material was provided by preparations obtained from a large number of normal guinea pigs (approximately 25 animals) stained by several different silver methods.

Many variations of standard silver stains for unmyelinated nerve fibers were tried in the course of this work. The procedure which we found most consistently useful is as follows:

1. Fix inner ear tissues by perilymphatic perfusion with 10% neutral formalin (stored over calcium carbonate). Allow the temporal bones to remain in formalin for at least 4 weeks at refrigerator temperature (2–6°C).

2. Remove temporal bones from formalin and perfuse with 5% trichloroacetic acid and leave in this solution 1–2 hours at refrigerator temperature.

3. Wash well in distilled water and dissect pieces of organ of Corti from the cochlea.

4. Place pieces in a staining solution made from 1 ml of 1% silver nitrate, 0.5 ml of 10% pyridine, and 48.5 ml of distilled water (pH of final solution 6.5 to 7.0) for 5 days at 37°C.

5. Transfer without washing to a reducing solution containing 10% sodium sulfite and 1% hydroquinone for 5 minutes at room temperature.

6. Wash well in 10% sodium chloride.

7. Transfer to 0.5% gold chloride for 20 minutes at room temperature.



8 Wash briefly in 10% sodium chloride

9 Place in fresh reducing solution (step 5) for 5-10 minutes

10 Wash with 10% sodium chloride and mount in glycerol

All the above solutions are prepared with distilled water

With this method both afferent and efferent neural elements in the inner spiral bundle region were stained against a suitably light background in whole mount preparations. In this material it was possible to survey large portions of the organ of Corti permitting small fascicles of fibers to be traced over long distances. It is recognized however that this technique like other silver methods presents certain disadvantages. In a given preparation not all the nerve fibers present become stained making quantitative studies unreliable. Also small fascicles composed of several fibers often stain as a single unit making it difficult to determine the precise course of individual fibers. On the other hand when the very dense innervation in the area of the inner hair cells is to be studied it is actually preferable that all fibers do not stain because of the simplification that results. Finally the emphasis in the present investigation is on clarification of the overall wiring plan in the inner spiral bundle region rather than on a determination of the exact lengths of individual fibers. For this purpose the above method was found to be useful.

## RESULTS

Previous workers have shown that in the region just distal to the habenula perforata and beneath the inner hair cells of the organ of Corti several different groups of nerve fibers are found closely packed together. Bundles of afferent fibers emerge from the habenula and distribute themselves in a predominantly radial fashion to the inner hair cells. Some afferent fibers course directly outward between the inner pillar cells to innervate the outer hair

cells. Other nerve fibers which experimental studies have indicated are of an efferent nature either enter the inner spiral bundle to course for variable distances between the inner hair cells or run straight out from the habenula into the tunnel of Corti and to the outer hair cells as upper tunnel fibers.

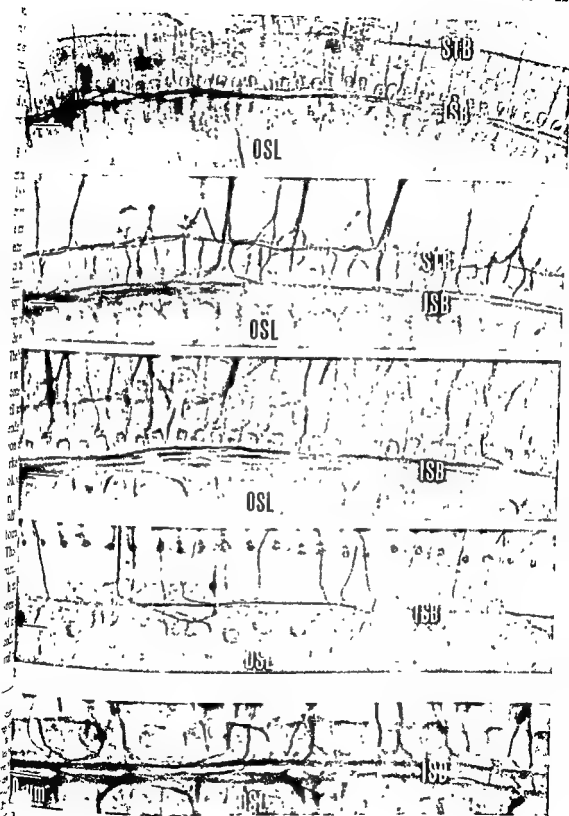
### General morphology of ISB

In normal animals the light microscope of this region is dominated by the inner spiral bundle. This bundle of unmyelinated fibers is located directly beneath the inner hair cells and not to be confused with either the reticular spiral bundle within the round window with the myelinated intralaminar spiral bundle prominent in the osseous spiral lamina and some other forms. Preparations of the ISB in various turns of the guinea pig cochlea are pictured in Fig. 2. The bundles in the upper basal or lower second turn of guinea pig cochlea and becomes slimmer in the upper turns until near the apex narrows down to a few slender fascicles. Toward the lower basal portion of the organ of Corti the ISB becomes markedly thicker. In the region of the basal hook very few long spiral fibers can be seen.

Especially in the basal half of the organ of Corti the ISB is frequently found to be divided into several sub-bundles. This division often takes the form of a separation into three discrete bundles which may rejoin after a short distance or in some cases separate for several hundred microns uniting again into a single bundle. Areas of such a divided inner spiral bundle are

Fig. 2. Photomicrographs of 1. stained inner spiral bundle (ISB) in the basal turn of the guinea pig cochlea.

ous spiral lamina. STB = spiral tunnel bundle. lower left represents 10  $\mu$ m in each case. Fig. 3. Photomicrograph of a stained inner spiral bundle (ISB) in the basal turn of the guinea pig cochlea. STB = spiral lamina.



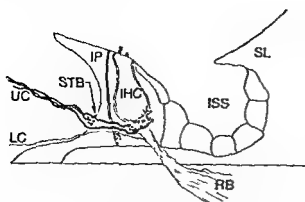


Fig 4 Diagrammatic cross section drawn from a silver stained specimen showing the inner spiral bundle beneath an inner hair cell (IHC). In this case the ISB is divided into three large fascicles separated by a radial bundle of fibers (RB) and an inner phalangeal cell (stippled). A lower tunnel-crossing fiber (LC) leaves the radial bundle to run directly into the tunnel without passing through any portion of the ISB. Upper tunnel-crossing fibers (UC) originate from both the ISB and spiral tunnel bundle (STB). IP inner phalangeal cell, ISS inner spiral sulcus, SL spiral limbus.

basal turn is shown in Fig 3. Each of the divisions contains fibers running in both directions and each gives off tunnel-crossing nerve fibers. Radial sections through these areas show that the ISB divides to pass on either side of the phalangeal cells and to surround the radial fascicles of fibers approaching the inner hair cells from the habenula perforata. This arrangement is diagrammed in Fig 4. In areas where such a separation is not apparent the fibers of the ISB are found more or less evenly distributed among the incoming radial fascicles and the supporting cell processes.

#### Brain stem lesions

The brain stem lesions made to interrupt the efferent innervation to the cochlea resulted in varying degrees of degeneration in the inner spiral bundle area. In the animals with longer post-operative survival times these variations in amount of degeneration were unexpected since in all 14 experimental animals the lesion site appeared to include all of the region occupied by the outgoing contra- and ipsi-lateral olivo-cochlear bundles—at least according to the majority of published accounts of the brain stem course of these tracts. In the ani-

mals with the more laterally placed lesions (shown in Fig 1) the degree of loss of fibers ranged from a slight reduction in appearance of 80% or more of the fibers normally present. This variation was seen in animals allowed to survive 2–4 weeks. Of the four cases in which the lesion had a medial position and extended slightly into the brain stem there was a total disappearance of long spiral fibers from the region and of upper tunnel-crossing fibers. Two of these animals had survival times of 10 days and two survived 14 days.

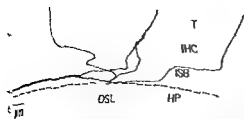
#### Patterns of fascicle distribution beneath the inner hair cells

Although the differing degrees of degeneration found in this series of animals were surprising, the preparations obtained provided material for the study of neural organization in the region occupied by the inner spiral bundle. It was possible to distinguish several nerve fascicles on the basis of their pattern of distribution in the region below the inner hair cells.

First there exist numerous heavily stained fascicles which emerge from the habenula and run directly through the ISB without taking a spiral course in the bundle. Fibers of this course to the outer hair cells as upper tunnel-crossers.

Other upper tunnel-crossing fibers take a short spiral course of 50  $\mu$ m or less in the ISB before making a right angle turn into the tunnel. These short spiral fascicles stain quite darkly and run either toward the base or the apex of the cochlea.

Two types of long spiral fascicles of varying size and staining intensity could be distinguished in the inner spiral bundle. One of these eventually turns outward to innervate the outer hair cells. The other remains within the ISB complex and ends there. It was difficult to trace slender fascicles coming from the base only a few fibers for several hundred  $\mu$ m without being able to discern a decrease in their diameter. At least some of the



Camera lucida drawing of a spiral fascicle splitting into several individual fibers, all of which cross the tunnel. A spiral fascicle was found in the apical turn coursing the apex of the cochlea. OSL, osseous spiral lamina; HP, habenula perforata; ISB, inner spiral bundle; IHC, nuclei of inner hair cells.

ent fibers must therefore extend the entire length of the fascicle. These small bundles of fibers were found running in both directions in the ISB and could often be followed for 100  $\mu$ m. Occasionally several fibers could be seen splitting off the parent fascicle to turn and cross the tunnel. The longest spiral fascicle that we were able to trace ran toward the base in the third and second turns and had a total course of 1.2 mm before it was lost in the tissue. It could not be determined if all the fibers of this fascicle crossed the tunnel, however, at least approximately 350  $\mu$ m from its entry into the ISB several fibers did split off as a separate bundle and run through the tunnel as upper spiral fascicles. Sometimes it was possible to trace these spiral fascicles to a point where they split up into smaller units, all of which crossed to the outer hair cells. One such fascicle in the apical turn is illustrated in Fig. 5.

Due to the fact that the silver method used in this work rarely stains the terminal portion of the nerve fibers, it was difficult to identify individual spiral fascicles which ended within the ISB region. However, a few fascicles were observed that did appear to end in this area. Others could not be followed for enough to determine their final destination.

Afferent fibers serving both the inner and outer hair cells are present in the inner spiral bundle region. The short fibers that go more or less directly to the inner hair cells comprise a larger group of afferents in the guinea pig. Generally, the afferent fibers destined for the

outer hair cells cross the tunnel of Corti near its floor. Some of these lower tunnel crossers split away from the fiber bundles immediately after their emergence from the habenula to run directly toward the floor of the tunnel, without passing through the ISB. One such fiber is diagrammed in Fig. 4. Others take a short, angular course through the inner spiral bundle of 10–30  $\mu$ m (in most cases) before entering the tunnel, the longest of these fibers that was observed ran 45  $\mu$ m in the ISB area. Fibers of this type are shown in Fig. 3, which was drawn from a preparation in which all long ISB fibers had disappeared. In the few cases in which all the ISB fibers had degenerated and there was suitable staining of the remaining afferent fibers we had the clear impression that the majority of incoming fibers terminated in the inner hair cell region. Only 2 to 4 fibers from each afferent fiber bundle could be found to cross the tunnel. No suggestion of afferent fibers which provide collaterals to inner hair cells before crossing to the outer hair cells could be found in these preparations.

A pattern of spiral fiber distribution which appeared to be confined primarily to the basal turn is illustrated in Fig. 7. In this portion of the cochlea bundles of fibers are often seen entering the organ of Corti and dividing into

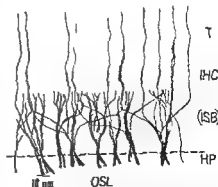


Fig. 6 Camera lucida drawing of afferent nerve fibers in the area normally occupied by the inner spiral bundle (ISB) in a preparation in which all ISB fibers have degenerated. Note that only a few fibers from each incoming radial bundle cross the tunnel space (T). OSL, osseous spiral lamina; HP, habenula perforata; IHC, nuclei of inner hair cells.



Fig 7 Camera lucida drawing illustrating the distribution of two nerve fascicles (A and B) in the inner spiral bundle (ISB) of the basal turn. Together the two main divisions

spiral lamina HP habenula perforata IHC nuclei of inner hair cells T tunnel of Corti

mediately into two fascicles which course in opposite directions in the inner spiral bundle. After a spiral course which is usually less than 100  $\mu$ m in length both fascicles turn outward to become upper tunnel crossers. These espalier like formations are frequently strikingly symmetrical with two limbs of roughly equal length. In some of these formations a central limb is present which goes directly into the tunnel as shown in bundle III of Fig 8. Also the limbs of adjacent bundles may join together to form a single tunnel crossing fascicle as seen in Fig 8.

Another pattern found in the upper turns as well as in the base is illustrated in Fig 9. In this figure several bundles can be seen entering the organ of Corti and dividing in such a way that one portion of each bundle proceeds directly across the tunnel while the other turns into the ISB. In a few cases long spiral fascicles originating in this way could be followed

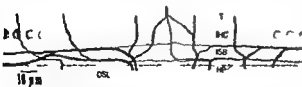


Fig 8 Camera lucida drawing illustrating a pattern of nerve fiber distribution seen in both the upper and lower turns of the cochlea. Several fascicles are shown entering the inner spiral bundle region (ISB) and dividing in such a way that one portion of each fascicle runs directly across the tunnel (T) while the other division enters the inner spiral bundle. The area shown is from the basal turn OSL osseous spiral lamina HP habenula perforata IHC nuclei of inner hair cells



Fig 9 Camera lucida drawing showing the distribution of several nerve fascicles emerging from the habenula perforata (HP) and entering the inner spiral bundle (ISB) in the basal turn. Fascicles such as A and B are shown taking a spiral course in the ISB. Interspersed fascicles which run across the ISB without any spiral course in the bundle are also shown. Fascicles A and B have a total distribution of 80-90  $\mu$ m in the ISB. OSL osseous spiral lamina IHC nuclei of inner hair cells T tunnel of Corti

100-200  $\mu$ m and then be seen to cross outer hair cells. The longest such fascicle could be traced to its entry into the tunnel 250  $\mu$ m toward the base in the basal turn.

## DISCUSSION

### ISB organization

One of the objectives of the present investigation was to study the organization of fibers in the inner spiral bundle throughout the guinea pig cochlea to determine whether differences in the peripheral innervation exist in the different turns. It was found that the similarities of organization from one turn to the next were more striking than the differences. The types of direct radial, short spiral and spiral fascicles described above were all closely intermingled in all turns of the Corti.

The overall size of the ISB is fairly constant through the middle turns, although in some preparations a slight increase in diameter is usually evident in the upper basal and second turns. The larger size of the bundle in this region appears to be due to an abundance of elements that give rise to tunnel-crossing fibers. This is in accordance with the well-established observation that

of efferent innervation to the outer hair is greatest in the lower portion of thelea (Fernandez 1951, Schuknecht et al., Engstrom et al., 1966, Spoendlin, 1966, h & Sjostrand, 1961) At both the apex extreme base of the organ of Corti the xer of fibers in the ISB is markedly red This reduction is most pronounced in basal hook where only a few, widely d fascicles can be seen These findings consistent with previous studies made several different species using acetyl nesterase staining where little positive ion was found in the ISB at the ex apical and basal ends of the cochlea knecht et al., 1959, Hilding & Wersall, Ishii & Balogh, 1968, Firbas & Wel ck 1970)

preparations stained by the ZIO method from et al (1966) have noted the occa l division of the ISB into several discrete les especially in the basal portion of the a pig organ of Corti In our silver stained al the bundle was often seen to split into or three large fascicles which might re ne almost immediately or, as was some the case, remain separate for several ed microns These splits appeared to oc relation to incoming radial bundles or to ingeal cell processes as illustrated in Fig ing the electron microscope, Smith (1961) ved bundles of ISB fibers dividing d phalangeal cells We found that the ISB t be divided into separate fascicles in any but this did occur most frequently in the half of the cochlea

the form of spiral fiber distribution il ated in Figs 7 and 8 was seen clearly only e basal turn In this region, bundles of e entered the organ of Corti and im ately bifurcated into fascicles which ran eposite directions in the ISB After a vari spiral course of up to about 100  $\mu$ m these cles crossed through the middle of the el spac to the outer hair cells The inner d bundle in the basal turn often appeared e composed of many of these units over

lapping one another and intermingled with very long spiral fascicles

In addition to those in the base, it was clear that many ISB fascicles in the upper turns also left the bundle to supply the outer hair cells —often after an extended spiral course under the inner hair cells It is, unfortunately not possible with the present method to make a reliable estimate of the proportion of ISB fi bers which eventually cross the tunnel However, it was obvious that this proportion is quite sizable the fibers of the inner spiral bundle certainly do not terminate exclusively in the inner hair cell region This point was also made by Fernandez (1951) in his now classical description of cochlear innervation in which he described fibers that run for 200  $\mu$ m or more in the ISB

### *Placement of lesions*

In view of the position of the brain stem lesions made in our experimental animals we were surprised to find large variations in the amount of degeneration that occurred in the inner spiral bundle region It seems unlikely that this was due to insufficient post-operative survival times since we found total degeneration of spiral tunnel bundle fibers (verified by electron microscopy) with survival times as short as 5 days (Wnght & Preston 1975) Different degrees of fiber loss in the ISB even after 3 to 4 weeks were found in several animals However in the cases in which the lesions were more medially placed (as shown in Fig 1) there was total disappearance of long spiral fibers in the ISB after survival times of 1–2 weeks

Thus the variability in degeneration was associated with the more lateral lesions which extended just into the dorsal aspect of the spinal tract of the trigeminal nerve The most plausible explanation for these findings appears to be that some of the exiting efferent fibers cross in a more ventral position through the nucleus and spinal tract of the fifth nerve rather than over the dorsal aspect of these structures Such fibers would be spared by

lesions that failed to penetrate deeply into the spinal tract of V. These fibers, however, would have been included in the more medial lesions which extended somewhat deeper into the brain stem.

Ross (1969, 1973) has described acetylcholinesterase-positive fibers crossing through the spinal tract of V, and other authors such as Osen & Roth (1969) and Rossi (1968) have included fibers in this position in their diagrams of the brain stem course of the efferent bundles. In Rossi's diagram it is specifically the ipsi-lateral bundle that is shown to cross in the more ventral position on its way to join the eighth nerve.

If this explanation for the results of the present study is correct we can then conclude that all long spiral fibers in the ISB of the guinea pig are efferent. This conclusion is in agreement with the findings of Spoendlin (1966) in the cat and of Smith & Rasmussen (1963) in the chinchilla. It should be noted, however, that the same results would be expected on the basis of Ross' concept of the parasympathetic innervation of the cochlea and that neither of the two types of lesions made in the present study is useful in deciding the question of the true origin and nature of efferent fibers supplying the inner ear.

### Afferent fibers

The limited observations we were able to make of afferent nerve fiber distribution in the absence of any inner spiral bundle fibers lend support to the conclusions of Spoendlin (1972) regarding the proportion of afferent fibers that end on the inner hair cells. His results indicate that the vast majority (about 95%) of all afferent fibers to the organ of Corti in the cat terminate on the inner hair cells. In our experimental preparations it appeared that only three or four fibers from each incoming bundle of afferent fibers crossed the tunnel to the outer hair cells. This observation matches almost exactly the electron microscopic findings of Iurato & Smith (1970) in the chinchilla cochlea.

In preparations in which all efferent had been eliminated we were unable to find giant fibers innervating the inner hair cells. The type Spoendlin (1972) has described in the cat. Also no suggestion of afferent fibers might have endings on both inner and outer hair cells could be found in our material. We were thus unable to find anatomical evidence for the interesting suggestion recently made by Nieder & Nieder (1970) regarding the existence of fibers that might be excited by the inner and outer hair cells.

Finally, it is of interest to note the types of afferent tunnel crossers existing in the inner spiral bundle. Those that take a short spiral course before actually entering the ISB and could therefore be contacted by efferent fibers of the inner spiral bundle. However, many afferent crossers enter from the radial bundles immediately and come through the habenula and run toward the tunnel without passing through the portion of the ISB. Such fibers cannot be identified in radial sections of the organ of Corti such as illustrated in Fig. 4, and therefore little possibility of synaptic contact between these fibers and those entering the inner spiral bundle.

### ACKNOWLEDGEMENT

The author wishes to thank Prof. Joseph E. H. for his thoughtful suggestions and encouragement throughout the course of this study.

The assistance of Verne T. Maulbetsch, Combs, and James W. Bruce in preparation of this manuscript is also gratefully acknowledged.

### ZUSAMMENFASSUNG

Die Organisation der Nervenfasern in der Langschnecke (*Nautilus*) wurde untersucht. Es wurde gefunden, dass alle langspiralen Fasern im inneren Spiralfaserbündel (ISB) in allen Querschnitten des Cortischen Organs sowohl kurze als auch lange Fasern enthält, die entweder zur Basis oder zur

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en Haarzellen. Operative Unterbrechung der ef-  
en Nervenversorgung im Hirnstamm fuhrt zur De-  
station aller ISB-Fasern. Nach der Eliminierung der  
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## NOISE AND THE RNA SYNTHESIS OF THE COCHLEA

### *Autoradiographic Studies*

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**Abstract** In cochlear basal turns of 22 guinea pigs with autoradiography, grain density was measured after injection of  $^3\text{H}$ -cytidine. Following high initial nuclear labelling a low incorporation in cytoplasm was observed even after 24 hours. Synthesis of ribosomal RNA in the organ of Corti seems to be slow. Low grain densities in the whole cochlea presuppose a blood-lymph barrier to cytidine. After white noise (85 dB SPL, 12 hours) were seen a more rapid incorporation into nuclei and after 24 hours an increased labelling of cytoplasm of ganglion cells, fibrocytes and inner hair cells, less of outer hair cells. This might be induced by increased blood flow, concentration of the precursor in the lymph, cell permeability or alterations of the cellular nucleotide pool. But a real stimulation of cochlear RNA synthesis seems to be the most likely. On comparing different turns a peak in grain density was found over nuclei of the organ of Corti after 1 hour in the 2nd turn. After 24 hours cytoplasmic labelling showed a decrease only in outer hair cells towards apical turns.

After the first report about the histopathology of noise damage by Habermann (1890), Wittmaack in 1907 assumed a "disturbance in the metabolism of the cell". Countless studies of enzyme histochemistry could not disclose the nature of this disturbance. Thus Wittmaack's random formula came to be cited almost literally up to the present day.

Many histochemical observations (Kluyskens, 1954; Beck & Michler, 1960; Vinnikov &

Titova, 1963; Nakamura, 1967, 1967) and measurements of nuclei (Neubert & Wüstenfeld, 1955; Wüster Halbfas, 1965; Anicin, 1968) assume the metabolism of the membranous cochlea promptly to loud noise. After intense stimulation, cytophotometric studies revealed a decrease in cytoplasmic RNA concentration in spiral ganglion cells (Hamberger & I, 1945; Hammer, 1958; Uno, 1958; O, 1960; Kon, 1964; Pakkenberg & Th, 1964; cf. Kraus & Gerlach, 1970). But both concentration and base composition remained biochemically unchanged (Ib al, 1965). In autoradiography with RNA precursors only occasional nuclei in Corti have so far been labelled (Kobayashi, Watanuki et al., 1968; Ruben, 1969; Bejdl & Krejci, 1953). Following application of streptomycin or kanamycin incorporation increased (Watanuki et al., 1968; Ruben, Kraus, 1973). Our knowledge of RNA metabolism of the cochlea is therefore compared with that of other organs. RNAs are key molecules in the regulation of cell metabolism. We have used autoradiography with  $^3\text{H}$ -cytidine in order to study the kinetics of RNA synthesis in the cochlea for 24 hours during rest and noise exposure.

This study was supported by grants from the Deutsche Forschungsgemeinschaft.

Abbreviations: RNA=ribonucleic acid; gd=grain density.



Fig 1 Autoradiogram of the organ of Corti from a guinea pig 1 hour after  $^3\text{H}$ -cytidine following noise of 85 dB. Only cell nuclei are labelled. Unstained phase contrast objective  $\times 40$  magnification  $\times 1040$ .

## METHODS

camera silens 22 juvenile guinea pigs weighing 110–150 g were offered only water and exposed to white noise of 35 dB SPL. At decapitation 1, 2, 3, 4, 8 and 24 hours after intraperitoneal injection of  $^3\text{H}$ -cytidine (10.0 Ci/mmol, specific activity 27.3 Ci/mmol, Amersham). Preceding injection one half of the animals were exposed to noise of 35 dB SPL for 2 hours (controls=C), the other half to noise of 85 dB (noise=N). A random noise generator, type 1402, Bruel & Kjaer was used, with a bandwidth of 20 Hz–20 kHz, linearity up to 20 dB. The noise level was checked with an audio frequency spectrum analyzer, type FNA, Rohde & Schwarz. After perfusion and fixation of the cochlea with glutaraldehyde for 2 hours, rinsing in buffer and embedding in Epon under standard conditions, 2–3  $\mu\text{m}$  sections cut with an ultramicrotome without previous decalcification, were covered with Agfa Gevaert emulsion NUC 7 15, exposed 9 months at 4°C, developed fixed and embedded in oil of identical refractive index (Kraus et al., 1973) without staining. Grain density (gd) over nuclei was measured with an incident light microscope photometer in a light field (Dormer et al., 1966, condensor

with fixed aperture of approx 0.3, condensor aperture diameter 6.7  $\mu\text{m}$ , measuring aperture diameter 4.8  $\mu\text{m}$ , 539 nm). Average reflexion values were checked against a standard and converted into number of grains/16  $\mu\text{m}^2$  by use of a calibration curve. Due to non linearity in the lower measuring range, the scale for nuclei is slightly distorted in Figs 2–4. Because of weak labelling we had to count through an eye-piece over the cytoplasm (phase contrast, oil, objective  $\times 100$  mesh ocular  $\times 20$  of the field area 16  $\mu\text{m}^2$ ). Measurements of cytoplasm of cells of tympanic lamina had to be omitted due to their irregular form. In stria epithelium dark cells were omitted. In ganglion cells only nuclei without nucleolar labelling were measured. For remaining cell types, measured values represent the mean for both nucleoplasm and nucleoli. Given mean values are derived from 3 petrous bones of 2 animals with at least 80–300 nuclei or 100–300 fields of 16  $\mu\text{m}^2$  in cytoplasm.

Statistical evaluation was performed for nuclei with U-test, for cytoplasm, after checking for Poisson distribution, with  $\chi^2$  test employing  $c_1$  transformation of mean values. Meaning in Figs 2–4:  $\square = P > 0.05$ ,  $+$  =  $P = 0.01$ – $0.05$ ,  $++$  =  $P < 0.01$ .

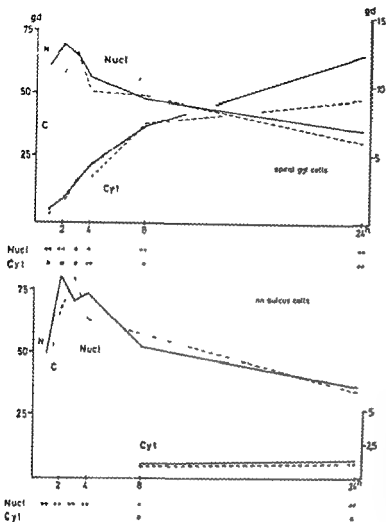


Fig. 2 Time course of grain density. Hours after ip injection. Ordinate: Grains/16  $\mu\text{m}^2$  at left,  $\times 5$  higher for cytoplasm at right. Controls = C (12 hours before to 24 hours after injection), N = noise (12 hours before injection to 24 hours thereafter 35 dB).

## DISCUSSION OF ERRORS

A non-specific binding of the precursor as a result of fixation seems to be insignificant and would have resulted already in labelling of cytoplasm after 1 hour. Control of specificity of grain density with RNase is not possible in Epon sections (cf. Kraus & Gerlach, 1970). The influence of  $\beta$ -self-absorption on the relation grain density to cytoplasm is probably weak, since a homogeneous permeation of cells can be expected in Epon sections (refractive index 1.54, Kraus, 1970, Kraus et al., 1973). Despite extremely long exposure, the labelling of background was only 0.34 grains/16  $\mu\text{m}^2$  for controls and 0.35/16  $\mu\text{m}^2$  after noise with a low standard deviation. For this reason no corrections for background activity have been made.

Differentiation between nucleus and cytoplasm in stria epithelium and between cytoplasm and intercellular space in fibrocytes of spiral ligament was difficult to make. The values are therefore probably too high in the first case, and too low in the second. Nuclei of cells of tympanic lamina were only those which were smaller than the background. They are therefore hardly countable.

## RESULTS

In autoradiograms after 1 hour, particularly of ganglion cells, are more extensively labelled than is nucleoplasm. The cytoplasm shows no incorporation (Fig. 1).

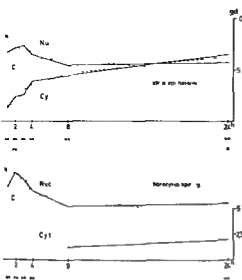


Fig. 2 For caption of Fig. 2

Schwann cells, all nerve fibres remain unlabelled for 24 hours

As the graphs show, we find in all cell types in the cochlear basal turn a rapid initial increase of  $^{3}H$  over nuclei, with a peak reached at about 3 hours, while only in cells of the stria and inner hair cells is this reached later on (Figs 2-4). Thereafter it decreases slightly biphasically up to 24 hours. In the cytoplasm the first grains appear at 2-4 hours. Their density increases up to 24 hours. Following noise, with the exception of the stria epithelium and inner hair cells, the nuclear peak is reached as early as after 2 hours and cytoplasmic labelling is enhanced at the time of maximal nuclear labelling. There are neither significant differences between  $^{3}H$  of different cells (Tab. I, cols 3-4), nor between noise and control (col. 6). In the stria the highest incorporation is observed after 24 hours in ganglion cells, the lowest in sulcus epithelium (cols 7-8). Following noise the highest percentage increase in incorporation after 24 hours is seen in ganglion cells, inner hair cells and fibrocytes, less in outer hair cells and stria (col. 9). The portion of maximal nuclear labelling which appears in the cytoplasm after 24 hours, amounts to

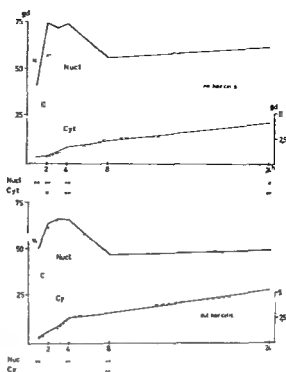


Fig. 4 For caption of Fig. 2

14-18% for ganglion cells and stria. Values for cells of Corti's organ are extremely low (cols 10-11).

As regards nuclear incorporation a significant difference is registered for different turns (Fig. 5). With the exception of outer hair cells, the whole organ of Corti shows a faster nuclear labelling in the 2nd turn. This maximum is raised after noise. After 24 hours cytoplasmic  $^{3}H$  shows a significant decrease from basal to apical turns only in outer hair cells, while other cells do not indicate a definite gradient.

## DISCUSSION

Precursors of ribosomal RNA, the bulk of cytoplasmic RNA, are synthesized in the nucleolus, those of messenger- and transfer RNA in the nucleoplasm. They are later transferred into cytoplasm for protein synthesis (cf. biochemical reviews). The kinetics of cochlear labelling with  $^3H$ -cytidine is in agreement. The

Table I Relation of grain density between different cell types of controls (C) and after

cytidine (col. 5) and at time of maximal labelling (col. 6)  
 Cols 7-8 Relation of gd in cytoplasm, 24 hours after cytidine Ganglion cells=100%  
 Col 9 Percentage increase of ...  
 cytidine  
 Cols  
 labelling

	Nucleus						Cytoplasm			Cytoplasmic
	Relation		Relation		Increase		Relation		Increase	Reb
	gd, 1 <sup>h</sup>		gd, Maximum		N to C (%)		gd 24 <sup>h</sup>		N to C	Cy-
	(% )		(% )				(% )		(% )	to
	C	N	C	N	1 <sup>h</sup>	At maxi-	C	N	1st-4th turn	back
	(1)	(2)	(3)	(4)	(5)	mum (6)	(7)	(8)	(9)	(10)
Ganglion cells	100	100	100	100	+79	+5	100	100	+34	14
Inner hair cells	72	67	97	107	+67	+16	35	33	+20	3
Outer hair cells	102	82	100	96	+47	0	55	43	+10	8
Deiters cells	117	91	104	105	+40	-6	24	20	+2	3
Claudius cells	98	74	104	98	+36	-5	18	15	+18	2
Inner sulcus epithelium	113	81	121	116	+25	0	12	11	+8	1
Cells of tympanic lamina	61 <sup>a</sup>	35 <sup>a</sup>	44 <sup>a</sup>	42 <sup>a</sup>	+5	0	-	-	-	-
Stria epithelium	80 <sup>a</sup>	60 <sup>a</sup>	70 <sup>a</sup>	57 <sup>a</sup>	+30	-15	69	53	+12	14 <sup>a</sup>
Fibrocytes of spiral hg	92 <sup>a</sup>	63 <sup>a</sup>	68 <sup>a</sup>	67 <sup>a</sup>	+29	+2	18	17	+30	4 <sup>a</sup>

<sup>a</sup> Cf. discussion of errors

same course of incorporation has been observed in a great number of cells in autoradiographic studies (for review Schultze, 1969). Thus RNA synthesis of the cochlea seems to be similar to other organs.

Here and previously (Koburg, 1961; Ruben, 1969; Richrath & Kraus, 1973) a very low labelling of the cochlea was seen in comparison with other organs. High doses of <sup>3</sup>H-cytidine and a very long exposure were necessary. This might be due not only to a blood-brain barrier (Schultze et al., 1972), but also to a blood-lymph barrier for cytidine. Unlike other organs, a very low and late labelling in cytoplasm has been seen in the cochlea. Similar findings were reported in biochemical and autoradiographic studies only of the retina (Koenig, 1971; Bondy, 1972). Thus a slow synthesis of ribosomes may be

typical for sensory receptors. In addition, absence of labelling of nerve fibres indicates slow axoplasmatic flow of RNA in the cochlea. Another cause for low labelling of cytoplasm may, however, be a relatively synthesizing activity in nucleoplasm. Fortunately, grain counts do not allow quantitative conclusions. Exact values of blood flow, kinetics of specific activity of cytidine in lymph, of cellular pools, and of the limiting transport of precursors through cell membranes are not available for the inner ear. Reutilization of precursors and the existence of a pool for nucleus and cytoplasm are still a matter of argument. However, following stimulation of noise, hair cells showed an increase, and only after prolonged stimulation a decrease in RNA concentration in the



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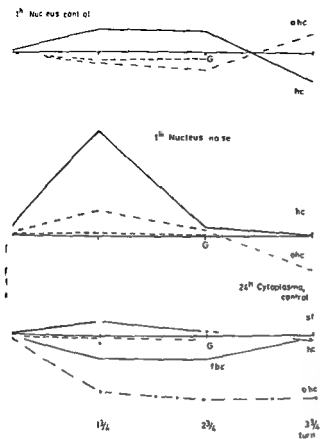


Fig. 3. Percentage increase or decrease of grain density in various turns compared with basal turn (=100%). ohc = inner hair cells, G = ganglion cells, st = stria epithelium, fbc = fibrocytes of spiral ligament.

photometric measurements (Ohhashi, Kon, 1964, cf. introduction). Since we used a very low SPL, a real stimulation of synthesis by noise is most likely here. The outer hair cells reacted more strongly than the inner hair cells. The absence of a correlation between cytoplasmic grain density and RNA concentration in both (Kraus & Doennig, 1969) can be explained by 'wastage in the ribo-cycle'.

The nuclear labelling of Corti's organ showed a significant peak in the 2nd turn. The concentration of the precursor in the middle turns is conceivable. It is missing at least, in the case of re-synthesis of pyridine nucleotides (Matschinsky & Mann, 1967). A differing hydrodynamic flow in middle turns has not been proven. The faster nuclear incorporation in the 2nd turn must be caused by local alterations of permeability, nucleotide pool, or RNA

synthesis. Unlike cell nuclei, cytoplasmic grain density of outer hair cells decreased towards apical turns. The same was observed by Meyer zum Gottesberge et al. (1965) for protein synthesis of cochlear ganglion cells. However, we found grain density in ganglion cells of different turns identical.

The synthesis of all enzymes, including those of known transmitters (Fonnum et al., 1973) is RNA-dependent. An important part of RNA synthesis in ganglion cells seems to be linked to the function of acetylcholine (Gisiger, 1971). Thus, a depletion of RNA during stronger stimulation may be one factor in the 'disturbance of cell metabolism', before the organ of Corti is mechanically damaged. Our investigation of the kinetics of RNA synthesis in the cochlea, as well as that of the breakdown of RNAs and acetylcholine (Schubert et al., 1970;  $\gamma$ -aminobutyric acid? Richrath & Kraus, 1974), agrees so far with the kinetics of noise trauma. An ex-



tion of RNA is more likely the cause of noise-induced permanent threshold shift (NIPTS) than of temporary threshold shift (TTS)

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## ZUSAMMENFASSUNG

Autoradiographisch wurde an 22 Meerschweinchen bis zu 24 Stunden nach Gabe von  $^3\text{H}$ -Cytidin bei weißem Rauschen von 35 dB SPL die Korndichte an Zellen der Basalwindung der Cochlea gemessen. Es kam bis 24 Stunden nur zu einer niedrigen Inkorporation im Cytoplasma. Im Cortischen Organ erscheint damit die Synthese ribosomaler RNA träge. Die niedrige Markierung der gesamten Cochlea läßt eine Blut-Lymph-Schranke für Cytidin vermuten. Nach Beschallung (85 dB 12 Stunden) fand sich prompt eine beschleunigte Inkorporation in Zellkerne mit erhöhter Korndichte nach 24 Stunden im Cytoplasma von Ganglienzellen, Fibrozyten und inneren Haarzellen, weniger in äußeren Haarzellen. Als Ursache kommen in Frage: Zunahme der Durchblutung, der Konzentration des Cytidins in den Lymphen, der Zellpermeabilität und Abweichungen im Nucleotid-Pool der Zellen. Am wahrscheinlichsten ist aber eine echte Stimulierung der RNA-Synthese. Beim Vergleich verschiedener Windungen fand sich in Zellkernen nach 1 Stunde eine beschleunigte Inkorporation in der 2. Windung, besonders nach Beschallung. Im Cytoplasma war nach 24 Stunden nur in äußeren Haarzellen ein Abfall der Markierung nach apikal nachzuweisen.

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## EVOKED RESPONSES FROM INFERIOR COLLICULUS AS AN INDEX OF HEARING THRESHOLDS IN GUINEA PIGS

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**Abstract** Bipolar electrodes were implanted in the inferior colliculus of 10 healthy guinea pigs and visual detection level thresholds of auditory evoked responses were obtained at 17 frequencies between 0.5 and 20.0 kHz. One microvolt isopotential curves from the round window for the same test frequencies were obtained in these animals. A comparison between the evoked response threshold curves and previous reports of the behavioral audiogram reveals a favorable correlation. The cochlear potential curve however appears to be a less efficient predictor of the audibility curve than the evoked response thresholds. The utility of evoked response audiometry from the inferior colliculus for quickly assessing auditory thresholds in the guinea pig is discussed.

A great deal is known about the morphology of the auditory system and the physiological mechanisms associated with hearing in the guinea pig. Relatively little, however, is known about hearing at the behavioral level in this species. This problem results from the difficulty in training guinea pigs to respond in the presence of sound. The application of classical conditioning (Horton 1933) and Preyer reflex (Gerstner 1942) procedures has met with only limited success. Moreover the catatonia-like behavior and low food requirements of the guinea pig have precluded many instrumental reward conditioning procedures. The recent use of behavioral suppression, however, has proved more successful and audibility curves have been obtained using response inhibition to

chill induced shivering (Andersen-Wedenberg 1965) to lettuce chewing (W. Murray, 1966) and to licking behavior (H. et al., 1971). Although these procedures have been successful in describing the absolute threshold of hearing, they have required investments of time in training and the problem of obtaining threshold information rapidly in the guinea pig has severely limited the use of this laboratory animal particularly in a situation in which changes in threshold sensitivity are important.

This study uses an objective physiological technique (evoked response audiometry) to assess auditory capacity in the guinea pig. This procedure provides a quick and valid index of threshold sensitivity which may serve as a valuable alternative to behavioral audiometry in many experimental situations.

### METHODS

#### *Subjects and preparation*

Ten adult colored guinea pigs weighing between 200-250 g were used. Each animal was anesthetized (sodium pentobarbital 10 mg/kg administered intraperitoneally) and a tracheal cannula was inserted. A head holder was used to secure the animal. The soft tissue was shaved from the skull from the midline to the external auditory canal. The superior part of the canal was incised about 3.0 mm from

ympanic ring. The skull was positioned so that the foramen ethmoidale and the center of the external auditory canal were in the horizontal plane. A small hole was made in the skull 2 mm posterior to the coronal suture and 2 mm to the right of the sagittal suture. The probe was situated directly over the right inferior colliculus (Rossner 1965).

After the experiment the head was rotated and the left auditory bulla was exposed auricularly. A small hole was drilled through the posterolateral aspect of the bulla to permit access to the round window membrane. All testing was conducted in a sound attenuated booth. A 60 W light bulb was placed above the preparation and the ambient air temperature was maintained at 37°C.

#### Stimulus

A sine wave output from an oscillator was fed into a tone burst by passing it through an electronic switch. The switch was gated at a 100 msec interval to produce 50 msec bursts with a 5 msec rise and decay time. The tone bursts were amplified and transmitted to a Teledynamics TDH-49 earphone. The stimulus frequency was monitored with a frequency counter and the intensity was set with a decade attenuator. When the a.c. cochlear potential responses were measured the same earphone was used but the electronic switch was permanently gated so that a tone was presented continuously.

Click stimuli were occasionally used and were produced by passing a 0.1 msec square wave pulse across the earphone.

The sound system was formed by sealing the earphone against a sound tube which was 4 mm diameter and 35 mm long. A 2 mm probe was inserted through the center of the sound tube to within 0.5 mm of the open end. The probe tube penetrated the sound tube at an angle of 45° about 20 mm from the open end. The tube extended 20 mm farther and was sealed against the probe microphone (Bruel & Kjaer 12.5 mm condenser microphone model 4134). The sound tube was calibrated by sealing a Bruel &

Kjaer 3.12 mm condenser microphone (model 4138) over the open end of the sound tube in the approximate position of the tympanic membrane of the guinea pig. The sound pressure level (SPL) at the probe microphone was corrected for the true SPL assumed to be at the animal's tympanic membrane. When the sound tube was positioned in the ear canal it was always placed as close as possible to the tympanic membrane. All sound levels detected by the probe microphone were converted to SPL at the tympanic membrane and expressed as dB re 20  $\mu\text{N/m}^2$ .

The signals detected at the electrode were amplified 1000 times (bandpass 1 Hz to 1 kHz) for the responses at the inferior colliculus, 100 Hz to 30 kHz for the responses at the round window. The responses at the inferior colliculus were displayed on an oscilloscope the sweep of which was synchronized with the tone bursts. The a.c. cochlear potentials were measured with a frequency sensitive voltmeter (General Radio model 1900A wave analyzer).

#### Procedures

The responses at the inferior colliculus were monitored first. The measurements were made with a bipolar concentric electrode which has a 0.5 mm sleeve and a 0.2 mm center shaft that extended 1 mm beyond the sleeve. The epoxy insulation was removed from the end of the sleeve and from the center shaft so that the vertical separation between the recording points was 0.5 mm. The electrode was lowered into the brain and auditory evoked responses were monitored as clicks were presented to the ear. The final position of the electrode in the inferior colliculus was determined on the basis of the amplitude and latency of the evoked response waveform. A response was considered satisfactory if it had an amplitude of 150–200  $\mu\text{V}$  and a latency of 1.5–1.0 msec to the first negative peak. These responses were typically found at an electrode depth of 2.5–3.5 mm below the brain surface. The preparation was allowed 10–15 minutes to stabilize before data were collected.

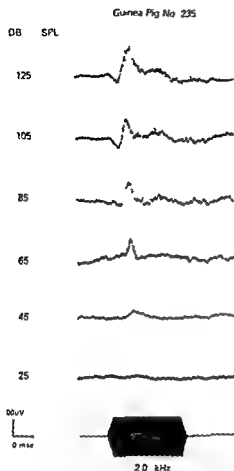


Fig. 1 Evoked responses from the inferior colliculus. A typical threshold sample for a tone burst in guinea pig No. 235. In this example the threshold was about 35 dB SPL.

A procedure similar to the method of limits was used to determine a visual detection level (VDL) threshold for the evoked responses at the inferior colliculus. Thresholds were obtained at 17 frequencies in successive 1/3 octave steps between 0.5 and 20.0 kHz. The SPL of the tone burst was set at a convenient supra threshold level (between 75 and 90 dB) and attenuated in 10 dB steps. With each step the oscilloscope was monitored to see if a response could be visually detected. This process continued until a SPL was reached which failed to elicit a detectable response. The SPL was raised 5 dB and the oscilloscope was monitored for a response. The threshold was defined as the SPL halfway between the highest sound level at which a response was not seen and the lowest sound level at which a response was observed. This procedure was repeated at all

test frequencies. A typical threshold is seen in Fig. 1 for a 20 kHz tone burst. As seen, the evoked response amplitude diminishes as the SPL is attenuated. In this example, the threshold was about 35 dB. After these thresholds were determined, a small lesion was made in the inferior colliculus by passing a current between the electrode for a few seconds. Histologic examination confirmed that the electrode had been in the inferior colliculus.

The animal was repositioned and the window membrane was visualized. The tube was repositioned, and a small silver electrode, made from 0.175 mm Teflon-silver wire, was placed on the round window membrane. Thresholds for the acoustic potential were determined at each frequency by adjusting the SPL until a response was obtained on the wave at 20  $\mu$ V. Data on the cochlear potential were collected from 8 animals.

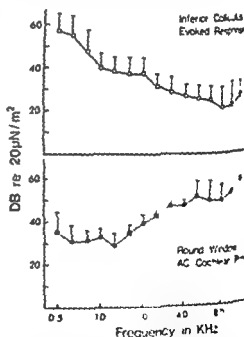


Fig. 2 Evoked response thresholds and cochlear potential thresholds. (Left) The acoustic thresholds at 17 test frequencies (successive 1/3 steps) are illustrated in the upper part of the figure. The bars indicate 1 SD above the mean. The  $\mu$ V isopotential curve of the AC cochlear potential is illustrated in the lower part of the figure. The bars indicate 1 SD above the mean.

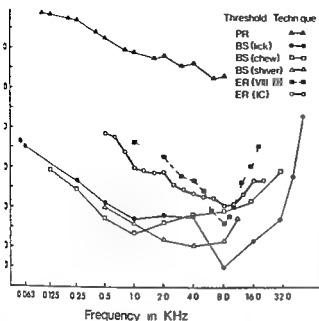


Fig 3 Comparison thresholds by various methods PR Preyer reflex by Gerstner (1947) BS behavioral suppression (lick) by Heffner et al (1971) (chew) by Miller & Murray (1966) and (shiver) by Anderson & Wedenber (1965) ER evoked response (VIII N) from the eighth nerve of hamster by Finck (1967) and (IC) from the inferior colliculus

## RESULTS

### Behavioral response thresholds

average VDL thresholds at each test frequency are presented in Table I and illustrated in the upper part of Fig 2. The vertical bars in Fig 2 indicate one standard deviation above the mean in the 10 subjects. Variability was small and fairly consistent at approximately 6.5 dB over all frequencies tested. The greatest sensitivity is at 8 kHz (20.8 dB SPL). There was a low frequency loss in sensitivity which increased approximately 9 dB/octave below 10 kHz. The high frequencies, above 10 kHz, show a loss in sensitivity of approximately 11 dB/octave.

### Cochlear potential thresholds

10  $\mu$ V isopotential thresholds recorded at round window are presented in Table I and

are illustrated in the lower part of Fig 2. The vertical bars in Fig 2 indicate 1 SD above the mean. The most sensitive frequency tested was 1.25 kHz (28.4 dB SPL). Above this frequency the threshold increases at approximately 8.0 dB/octave.

### Comparison of threshold methods

The behavioral thresholds reported for the guinea pig are reproduced in Fig 3. The Preyer reflex data reported by Gerstner (1942) demonstrate high threshold levels typical of this procedure. The three absolute thresholds obtained by suppression procedures (Anderson & Wedenber 1965; Miller & Murray 1966; and Heffner et al 1971) are very similar although Heffner et al (1971) showed greater sensitivity with suppression of licking at 8.0 kHz than was

Table 1 Mean thresholds (dB SPL) in guinea pigs (frequency in kHz)

nd ng	Frequency (kHz)																	
	0.5	0.63	0.8	1.0	1.25	1.6	2.0	2.5	3.15	4.0	5.0	6.3	8.0	10.0	12.5	16.0	20.0	
nor	56.3	54.5	47.6	39.8	38.0	37.7	37.4	31.3	28.3	26.3	25.7	24.1	20.8	21.8	26.0	33.2	33.2	
Acus	35.3	31.4	31.9	32.7	28.4	34.3	39.5	42.5	47.0	47.0	51.9	49.7	48.6	57.6	58.3	62.1	62.0	
Al																		
low																		

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## NORMAL CRITICAL BANDS IN THE CAT

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Critical bands in the cat were measured by a  
aural psychophysical method. Pure tones were  
noise of variable bandwidth but constant total  
geometrically centred on the test tone, the point at  
the masked threshold began to fall as the masker  
dth was increased estimated the critical band.  
At 2 kHz the critical bandwidth was also measured  
wideband masked thresholds of both tones and  
f variable bandwidth: this produced the same result  
first method. The measured critical bandwidth was  
r than previously published values of the effective  
ndths of single fibres of the auditory nerve. The  
do not fit in with the commonly accepted theory  
critical band represents the resolution of the coch

an psychophysical experiments with  
lex stimuli have shown that at any one  
ency the auditory system possesses a  
defined bandwidth of resolution. This  
width is known as the critical bandwidth  
s constant over many different auditory  
(Feldtkeller & Zwicker, 1967, Scharf,  
1. The same critical bandwidth is for in-  
e thought to govern the masking of sig-  
by broadband noise, the thresholds and  
ned loudness of complex sounds, and  
tainable limits of both frequency and  
oral resolution (Scharf, 1970). It is gener-  
accepted, although only on indirect evi-  
e, that the mechanism of critical bands is  
ted in the cochlea itself, and that the criti-  
bandwidth equals the bandwidth of the  
anical resolution of tones in the cochlea.  
ever there has hitherto been no direct

test, as critical bands have not been measured  
directly in any species for which measures of  
the resolution bandwidth of the various stages  
of the auditory system have been available.  
The mechanical resolution of the cochlea has  
in any case only been measured at high sound  
intensities where, in man at least, the critical  
band becomes very broad. If critical bands  
were indeed set up in the cochlea, one might  
expect the critical bandwidth to be reflected in  
the resolution bandwidth of the cochlear out-  
put, that is in the bandwidths of single fibres  
of the auditory nerve. Their effective band-  
widths, or bandwidths of their equivalent rec-  
tangular filters, should be equal to the effec-  
tive bandwidth of the critical band measured at  
the same intensity and frequency. In the ex-  
periments described here, critical bands have  
been measured in the cat by a behavioural  
psychophysical technique. As a direct test of  
the cochlear origin of critical bands the band-  
widths were compared with the effective  
bandwidths of single fibres of the auditory  
nerve, measured by Evans & Wilson (1971).

### METHODS

#### *Estimation of critical bandwidth*

A pure tone stimulus was masked by a noise  
band of constant total power but variable  
bandwidth, geometrically centred on the test  
tone. Pure tone maskers were not used as the  
auditory nerve does not necessarily behave  
linearly in combinations of pure tones even at

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threshold, whereas it seems to do so for broadband stimuli at low intensities (Sachs & Kiang, 1968; Evans & Wilson, 1971). For masker bandwidths smaller than the critical band the masked threshold should be independent of masker bandwidth if the results of Greenwood (1961*a*) apply, and for masker bandwidths larger than the critical band the masked threshold should decline in inverse proportion to masker bandwidth, that is, at 3 dB per octave of masker bandwidth. This model was tested and found to be adequate (Results section). The relation between the masked tone threshold and masker bandwidth both on logarithmic scales, was therefore fitted with a function of two intersecting straight lines, one of zero slope for small bandwidths, and one of slope  $-3$  dB per octave for larger bandwidths, using the method of least squares. The intersection was at the estimated critical bandwidth. Although the method is only strictly applicable if the critical band function is rectangular in shape, it in fact closely estimates the effective bandwidth of the critical band over a wide variety of shapes. If for instance the critical band function is of Gaussian form, the method produces a value of the effective bandwidth which is overestimated by between 10 and 15%, for the bandwidths actually used.

### Stimuli

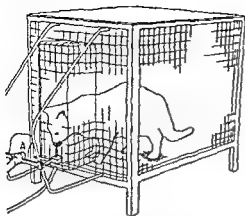
The output of a Solartron type CO546 oscillator was gated with an FET gate giving a signal which was a series of tone pips each of rise and fall time 22 msec, duration 169 msec, and repeated at 3/sec. Bandpass masking noise was produced by modulating lowpass filtered thermal noise (low frequency cutoff 20 Hz) with a carrier sinusoid in a balanced modulator. The high frequency cutoff slope of the lowpass filter was 12 dB per octave. The slopes of the output band of noise depended on the modulation frequency and the noise bandwidth. For instance the lowpass cutoff slope modulated in frequency to ten times its original frequency will become a cutoff of 120 dB

per octave. All harmonics in the band were 60 dB or more below band, except the third harmonic, 39 dB below. An attenuator-gated pass filter cutoff control ensured 1% power to the modulator stage was within  $\pm 0.2$  dB whatever the band. Accuracy of the final output band was checked in three ways: (i) The noise spectrum was checked in 10 bands (Brüel & Kjær bandpass filter 1612). (ii) The output of the balancer stage was checked with a waver with 10 Hz resolution (Federfic 'Ubiquitous Spectrum Analyser'). The technique of estimating the effective width of a filter by the method above for measuring critical bandwidth was applied to measuring the bandwidth of known width. The total noise was 30 dB SPL at 1 and 2 kHz, 10 dB SPL at 8 and 16 kHz. The highest intensity was used at the high frequency cat's tongue, in the licking task it generated some noise.

One of three transducers and amplifiers was used, depending on the test frequency. At 2 kHz and below, a cone speaker was adequate. A Goodmans Axiom 100 was used, driven by a Leak TL12 power amplifier followed by a 40 dB power attenuator. At 8 kHz an Ionofane ionic speaker was used. At 16 kHz a type 4132 1-inch Brüel microphone with grid removed was used as sound source, with 200 V polarisation, driven at 2 V RMS maximum. Sound level was calibrated with Brüel & Kjær half-inch quarter-inch condenser microphones 4133 and 4135, grids removed, and a Brüel & Kjær type 2606 Precision Sound Level

### Measurement of thresholds

Cats were tested in a sound proof room with sound absorbent inner surfaces. A shuttle box was found to be satisfactory for measuring thresholds at 2 kHz and below. The box was 60 cm high, 60 cm wide and



Apparatus for the measurement of thresholds at frequencies. The sound transducer is mounted at A. The cat licks a small dish for milk which is supplied through a tube. Wire baffles in the cage ensure that the cat with its head in only one position. Stimuli are only presented while the cat is licking and so its head is in the sound field.

divided in two by a barrier 4 cm high floor, through which shocks were given, consisted of bars 1.7 cm apart. Alternate bars were connected to one polarity of the shock generator. The current applied to the cat was usually 3 mA, although because the bars were close together most of this probably flowed on the surface of the foot pads. The speaker was mounted axially 2 m above the

The warning period was 15 sec long and the cat to detect was punished on a variable schedule, the ratio varying from 0.5 to 1.0 depending on the cat. Above 2 kHz, the free field method was found to be completely inadequate. In most of the sessions the responses were too inconsistent to allow a threshold to be assessed, and if one could it was much higher than expected. Instead, conditioned avoidance during the experiment was used. Stimuli were only delivered while the cat was licking for milk, and so its head was fixed in front of the high frequency sound source (Fig. 1). The field was even to  $\pm 2$  dB over the space occupied by the cat's head, covering the one-octave band (at 8 kHz) or the octave band (at 16 kHz) containing the stimulus. Wire mesh barriers in the cage ensured

that the cat could lick only with its head in one position. The cats were food deprived and fed entirely on the 120 ml of evaporated milk (Carnation Milk, Carnation Foods Co. Ltd., England) obtained each day in the apparatus. Licking the dish, which was 2 cm diameter, initiated a trial. Once the trial had begun, milk was pumped into the dish at the rate of 3 ml/min while the cat was licking. On 1/3 of the trials the stimulus was switched on after 5 sec if the licking was steady. In this case the first lick between the 15th and 20th sec of the trial was punished with a shock on a variable ratio schedule, the ratio used varying from 1.0 to 0.3 depending on the cat. Cessation of licking in the danger period showed that the cat had heard the stimulus. The other 2/3 of the trials were dummies and used to assess the false positive detection rate. Thresholds were measured by tracking with a final step size of 1 dB as described by Pickles & Comis (1973). The criteria they described for an equilibrium threshold series were used here, with one exception. When measured by the licking technique, some animals' thresholds fell steadily throughout the session, presumably due to declining hunger. In these cases the threshold was assessed over a fixed number of trials in the session.

### Subjects

All cats were monaural; the contralateral middle ear apparatus having been destroyed and the middle ear cavity filled with bone cement. All except cat 32 had cannulae implanted over the operative cochlear nucleus at some time during testing, as described by Pickles & Comis (1973). In all, 76% of thresholds were obtained in cats after implantation. Measurements before and after the implantation as described in the results section showed that cannulation did not in fact affect the critical bandwidth. The cats' absolute thresholds were comparable with those previously described (Elliott et al., 1960; Neff & Hind, 1955; Igarashi et al., 1972).

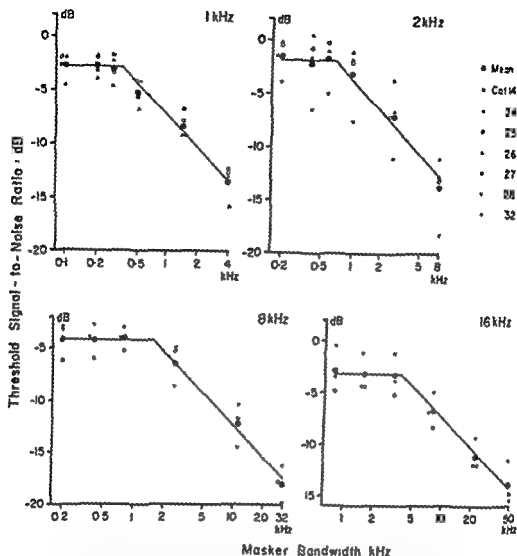


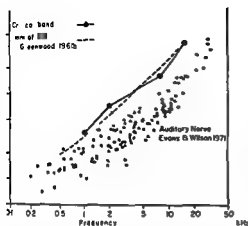
Fig 2 Masked tone thresholds for different bandwidths of masking noise at the four test frequencies. The masking noise is of constant power but variable bandwidth geometrically centred on the test frequency. The mean

thresholds have been fitted by lines of zero slope -3 dB per octave. The corner is at the critical bandwidth.

## RESULTS

Fig 2 shows each cat's mean thresholds in all bandwidths of noise for the four test frequencies. The mean thresholds taken over all the cats have been fitted with the expected two-part function, namely, a line of zero slope inside the critical bandwidth and one of slope -3 dB per octave outside the critical bandwidth. Around these mean values considerable scatter of individual cats' thresholds is apparent. Closer inspection however shows that each cat's thresholds are much more consistent within themselves than this scatter

might at first suggest. For instance thresholds of cat 32 at 16 kHz are consistently higher than the mean but are well fitted by a two-part function similar to that fitted to the mean. Presumably this cat has a critical bandwidth similar to the mean but a threshold signal-to-noise ratio similar to cat 25 at 16 kHz has a threshold which is consistently lower than the mean but which is fitted by a two-part function of approximately the same critical bandwidth. Between cats there may be a range in estimated critical bandwidth rather than in threshold level.



3 The critical bandwidth at the four test frequencies is clearly greater than the effective widths of fibres of the cat auditory nerve which were tuned by comparing the fibres' thresholds to tones their thresholds to wideband noise (Evans & on 1971). However the critical bandwidth corresponds closely to 1 mm of the cat basilar membrane as fitted by Greenwood (1961 b).

ratio. The greatest range is shown at 8 kHz where the thresholds of cat 28 are closely fitted by a two-part function giving a critical bandwidth of only a quarter of that of cat 25. In the same population of cats was not necessarily used for each test frequency, comparison between frequencies must therefore be made with caution.

Analogy of the sort described above will have only a small effect on the fit of the mean thresholds to the expected two-part function. The fit of the data to the function can be assumed to be adequate, then the method can be expected to be working and the position of the corner can be used to estimate critical bandwidth. Different functions might however have been expected, in particular slopes of not more than  $-1.5$  dB per octave have been sometimes reported for the relation between threshold and bandwidth within the critical band (Hamilton 1957). In order to test this possibility, two-part regression lines were fitted by the method of least squares. The mean slope for narrow bandwidths was  $-0.07$  dB per kHz (S.D.  $0.5$  dB per octave, 10 d.f.). This is not significantly different from zero slope

but was significantly different from a slope of  $-1.5$  dB per octave ( $p < 0.05$ , 2-tailed). For wide bandwidths the slope also agrees with the value expected, the mean fitted slope was  $-3.04$  dB per octave (S.D.  $0.16$ , d.f. = 15), not significantly different from the expected  $-3$  dB per octave. The expected function moreover, provides an adequate account of the variance in the original data. The variance of each point fitted, calculated from the variance within the raw data, was  $0.274$  (dB scale, 104 d.f.), once the mean of each cat's thresholds was adjusted to the same grand mean. That expected from the variance of the mean points around the fitted lines was  $0.247$  (16 d.f.). The difference is not significant ( $F$ -test). The original two-part function seems therefore to be an adequate description of the data and the position of the corner can be expected to estimate the critical bandwidth.

Fig. 3 shows the value of the critical bandwidth as a function of test frequency. The standard deviation of each value calculated from the fit of the data to the expected two-part function is between 5 and 15%; other unknown errors may of course be larger. As a function of test frequency, the critical bandwidth increases with a mean slope of  $0.80$  when plotted on log-log axes. Fig. 3 also shows the relation between the measured critical bandwidth and two measures of cochlear function. The critical bandwidth is clearly greater than the mean of the effective bandwidths of single auditory nerve fibres. Direct comparison is possible as the measures are made in a way which is substantially independent of the shape of both the tuning curve and the critical band resolution function. The difference is approximately 2.5 times and is constant over the whole frequency range. Furthermore intriguingly the cat critical bandwidth corresponds closely to the 1 mm of basilar membrane originally suggested by Greenwood (Greenwood 1961 b).

One objection to comparing effective bandwidths of auditory nerve fibres with those of the critical band is that the two were measured

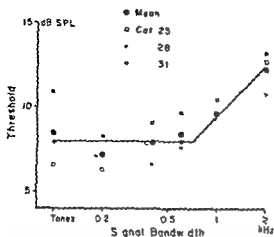


Fig. 4. Wideband masked thresholds of both tones and noise bands centred on 2 kHz. The noise signal is of constant power but variable bandwidth; the tone threshold is the mean of the thresholds of five tone signals evenly spaced between 1.70 and 2.30 kHz. The mean thresholds have been fitted by one line of zero slope and another of slope +3 dB/octave. The corner is at the estimated critical bandwidth which is very close to that estimated from the data in Fig. 2.

at different intensities and by different methods. Evans & Wilson (1971) measured the fibres' effective bandwidths by comparing their absolute thresholds to pure tones with their absolute thresholds to wideband noise. Here masked tone thresholds were measured in different bandwidths of masking noise at 30 to 50 dB above the cats' absolute threshold. An attempt was therefore made to measure critical bands by a method corresponding to that of Evans & Wilson, namely by comparing the absolute thresholds of tones and of noise of different bandwidths. When used psychophysically, however, this method will only work if used at the most sensitive part of the audiogram. There is a danger that otherwise as the signal bandwidth is widened the signal will be detected in a different frequency range where the animal is more sensitive. As this was not necessarily possible the audiogram was flattened by wideband masking noise at an intensity which in fact raised thresholds from a mean of  $-14$  dB SPL to  $+8$  dB SPL. The masking noise was of constant power per octave and the noise signals were centred arithmetically at 2 kHz. Five frequencies of

pure tone signal were used spaced evenly within the expected critical band at 1.70, 1.85, 2.0, 2.15 and 2.30 kHz. Fig. 4 shows the relation between threshold and stimulus bandwidth. A two-part fit of slopes zero and +3 dB per octave fitted to the data estimates the critical bandwidth at  $708 \pm 75$  Hz, in close agreement with the value of  $700 \text{ Hz} \pm 98 \text{ Hz}$  obtained by the earlier method. This suggests that the critical bandwidth does not change with intensity; moreover that it seems to be the same as measured by the spectral resolution of a narrowband stimulus in wideband masking or by the spectral integration of a wideband stimulus. Similar behaviour has been found in human beings (Gissler, 1954; Feitch & Zwicker, 1967). Direct comparison between the neural and psychophysical data is not yet possible.

The overall mean determinations are given in Table 1.

At some time during testing eight of nine cats used had cannulae implanted in the operative cochlear nucleus as described by Pickles & Comis (1973). Drugs known to affect specifically the centrifugal innervation of the cochlear nucleus, were applied to the surface of the cochlear nucleus in order to study the influence of the centrifugal innervation on the critical bandwidth. These results will be described separately. The post-operative after-effect of cannulation and injection on critical bandwidth was assessed by comparing the critical bandwidth at 1 kHz before and after all intervention. The normal critical

Table 1. Summary results of the critical bandwidth, critical ratio and signal-to-noise ratio within the critical band.

Frequency (kHz)	1	2	3	4
Critical bandwidth (kHz)	0.15	0.70	1.00	1.10
10 log <sub>10</sub> critical bandwidth	1.4	14.1	11.5	11.5
Critical ratio (dB)	2.6	26.3	24.5	24.5
Signal to noise ratio (dB)	2.8	2.2	4.2	

width was  $3.45 \text{ Hz} \pm 0.076 \text{ log units (4 df)}$  that after all intervention was  $371.0045 \text{ log units (4 df)}$ . The increase is 2 log units which is not statistically significant. If the difference between the neural and psychophysical resolution bandwidths were to be ascribed to cannulation, the relation would have to have been having effect which, on the basis of the above data, could be expected to arise with a probability less than 0.0005 (1 tailed). It is very unlikely, therefore, that the observed difference between the neural and psychophysical resolution bandwidths was due to the cats' being normal.

## DISCUSSION

Previous measurements of the cat's sensitivity to signals in noise have been confined to determinations of the masked threshold in bands of masking noise wider than the critical bandwidth (Fletcher 1963). This produces a measure which has been called the critical ratio (Fletcher et al., 1957) and is calculated as the level of the signal at masked threshold minus the power per unit bandwidth of the masking noise. The critical ratio can be calculated in the present experiment from the data in Figs 2 and 4 and is shown in Table 1. At 1 and 2 kHz it averages 1.5 dB greater than that obtained by Watson, and at 8 and 16 kHz it averages 4 dB less. The discrepancy at the higher frequencies may be caused by differences in technique. In the present experiment special care was taken that the sound field should be uniform at these frequencies and that the cat should be in a constant position whenever the stimuli were presented. A free-field shuttle box and cone speakers as used by Watson (1963) produced much higher thresholds at the high frequencies. In fact, Watson made some measurements with an omnidirectional loudspeaker, which should have produced a more even sound field. He obtained thresholds which were closer to the present results but decided to ignore them as they did

not agree with his other measurements. They may, after all, have been the more accurate.

The sensitivity to signals in wideband noise is determined by two factors. One is the bandwidth of the critical band filter, or in other words the noise power from which the signal must be discriminated after filtering by the critical band mechanism. The other factor is the signal to-noise ratio at which the signal can be detected in the resulting band of noise. In man, the signal to noise ratio within the critical band is commonly thought to be -4 dB above 1 kHz (e.g. Scharf, 1970), although not all measurements agree with this (Greenwood 1961a). In the present experiments it is a little greater, with a mean value close to -3 dB.

A second factor which in man is thought to be related to the critical bandwidth is frequency discrimination. It has been suggested that the frequency discrimination limen is proportional to the critical bandwidth (Fletcher, 1940; Zwicker et al., 1957). The present results, together with the frequency discrimination data of Elliott et al. (1960) suggest that this may not be so in the cat. The measured critical bandwidth is 40 times the frequency limen at 1 kHz, 49 times at 2 kHz, but declines to only 16 times at 16 kHz. There is a similar lack of proportion between the cat frequency discrimination threshold and the auditory nerve fibre effective bandwidths. Lack of proportion between frequency discrimination and the critical ratio has also been found above 1 kHz in the parakeet (Dooling & Saunders 1975). Moore (1974) using a frequency discrimination method which reduces intensity cues, has found similar results in man. The generally accepted proportionality in man may not therefore be of fundamental significance.

A third measure which in man is believed to be related to the critical bandwidth is position on the basilar membrane. It was suggested originally by Fletcher (Fletcher, 1940) and later by Greenwood (1961b) that frequencies separated by a critical bandwidth were represented by a constant distance apart on the basilar membrane. Greenwood, on the basis of

Békésy's experiments (Békésy, 1960), showed that this was so in man, and that the distance was 1 mm. He suggested that this would be true in other species. This in fact seems to be true for the cat (Fig. 3). Whether it is just a coincidence or stems directly from the mechanism of the critical band will be discussed below. Fletcher's view, and one that has been generally held since, was that the critical bandwidth represented the mechanical frequency resolution of the cochlea. The relation between the critical band and constant distances of the basilar membrane supported this view, although it was contradicted by Békésy's measurements on human cadavers which showed that the mechanical frequency resolution of the cochlea was up to six times poorer than that of the critical band (Békésy, 1960). Recent measurements have shown that the basilar membrane is in fact much more sharply tuned than previously thought (Rhode, 1971). However, measurements of the basilar membrane are not yet reliable enough at medium and low intensities to make comparison with the critical band worthwhile. A second measure of cochlear frequency resolution which can be obtained under rather more physiological conditions is the frequency response of single fibres of the auditory nerve. Two components of the frequency response can be identified. A sharply frequency selective response to stimuli which has been called the 'tip' of the tuning curve, namely, within an intensity range of about 40 dB above the fibre's lowest threshold and over a narrow frequency range around it. Within this range the fibre seems generally to behave linearly to broadband stimuli and hence is of constant effective bandwidth from threshold to about the 40 dB limit above it (Evans & Wilson, 1971; de Boer, 1969; Møller, 1970; de Boer, 1971). At higher intensities in the 'tail' of the tuning curve, the effective bandwidth becomes much greater (Kiang et al., 1965). In human beings the critical band correlates with this behaviour, although the intensity ranges are different. From threshold to some 70 dB above

the critical band is of constant width (Gates, 1954; Feldtkeller & Zwicker, 1967). At higher intensities the critical band seems to be considerably wider. If therefore comparison is to be made between the critical bandwidth and the auditory nerve fibre effective bandwidth, it might be appropriate to begin by comparing the low intensity critical bandwidth with the effective bandwidth of the sharply-tuned part of the tuning curve. Such a comparison is shown in Fig. 3 and shows that the critical bandwidth is in fact greater. In fact the discrepancy may be greater than is immediately apparent. The critical bandwidth in this task can be expected to be governed by the fibres that are most sensitive to masked tones. Those fibres will be the narrowest effective bandwidths. Figure 3 shows that the critical bandwidth is approximately four times greater than the effective bandwidth of the narrowest neurone. At high intensity, the tail of the tuning curve has a much greater critical bandwidth. Although values of the effective bandwidth of the tail have not been published, the data of Kiang & Morrell (1958) suggests that the 3 dB bandwidth of the tail dip in the tail can be as wide as 15% of the frequency. This is several times greater than the critical band on logarithmic scales. A further difficulty arises from the observation of Watson (1962) that the tail of the tuning curve for signals in noise is constant over a 60 dB range above absolute threshold, this range also spans both the sharply-tuned and broadband portions of the tuning curve.

Some nonlinear effects with broadband stimuli have been described in the monkey auditory nerve by Ruggero (1970). He found that widening a band of noise around a neurone's characteristic frequency suppresses its responsiveness. If such a band were to determine thresholds in the present measurements, one might expect the defined masked thresholds to be different from 100 per octave for maskers wider than the critical band. This is not so in the present experi-

has it been described in man. If the supposition occurred with noise bands narrower than the critical band, one might not expect the threshold of a complex stimulus to be independent of its bandwidth, if its width is narrower than the critical band. Such independence has been shown in human beings (Zwicker 1954). The evidence at present does not support nonlinear mechanisms in the auditory nerve as being the basis of the critical bandwidth, although further experiments may change this.

The discrepancy between the critical bandwidth and the bandwidth of the auditory nerve fibres indeed exists, it might be suggested that it arises from the different conditions in which the observations were made. The fibres were recorded in the anaesthetized cat, whereas the critical bandwidth was measured in the unanaesthetized, attentive, animal. The activity of the olivocochlear bundle is thought to be too low in even the decerebrate cat to affect the afferent fibres (Fex, 1965), whereas it might be greater in the intact, unanaesthetized cat. It is known that its activity increases the bandwidth of auditory nerve fibres by selectively inhibiting the sharply tuned tip (Wiederhold, 1970). However lesions of the crossed component of the olivocochlear bundle do not seem to affect the cat's ability to detect signals in noise, certainly they do not remove it (Galambos, 1960; Trahiotis & Elliott, 1970; Igarashi et al., 1972). The activity of the middle ear muscles in the unanaesthetized cat might also, by changing the frequency response of the middle ear, considerably increase the minimum measurable resolution bandwidth of the auditory system. However that the resolution, as shown by the auditory nerve fibre bandwidths, does not in fact vary with anaesthetic is suggested by Simmons & Linch (1968). They recorded at a depth they thought was the same fibre in both anaesthetized and unanaesthetized cat and found very much the same bandwidth. These facts and the present observations lead to the doubt on the simple identification of criti-

cal bandwidth with auditory nerve resolution, and hence with cochlear resolution. This follows, for if the mechanical resolution of the cochlea indeed determined the critical bandwidth, correlations should be observable in the responses of the auditory nerve, as it is those that influence the higher centres. The correlation of the critical bandwidth with 1 mm of basilar membrane may therefore just be a coincidence. Two alternative hypotheses are possible. One is that the resolution of the cochlea after all determines the critical bandwidth, but that the unanaesthetized, attentive, animal uses in the discrimination of complex stimuli some properties of the auditory nerve which are different from those currently understood. Another hypothesis is that the critical bandwidth reflects integrative processes at higher centres. Whether or not the critical bandwidth can be identified with processes in the cochlea, or indeed with any one stage of the auditory system, can only be answered by further physiological and psychophysical investigations in animals.

## ZUSAMMENFASSUNG

Die Breite der Frequenzgruppen bei der Katze wurde durch eine psychophysikalische Verhaltensmethode gemessen. Reine Töne wurden durch Geräusche von variierender Bandbreite aber konstantem geometrisch auf den Prüftönen zentriertem Totalschalldruck verdeckt. Der kritische Frequenzabstand wurde durch den Punkt bestimmt an dem die Prüfschwelle niedriger wurde, während die Verdeckungsbandbreite erhöht wurde. Bei 2 kHz wurde der kritische Frequenzabstand auch durch die durch Weitband verdeckte Prüfschwelle für Töne und Geräusche von variierender Bandbreite bestimmt. Dieses ergab ein mit der ersten Methode übereinstimmendes Resultat. Der auf diese Weise bestimmte kritische Frequenzabstand war größer als die bisher publizierten Werte für effektive Bandbreiten von Faserfasern des Gehörnerven. Die Resultate stimmen daher mit der allgemein akzeptierten Theorie nicht überein, dass der kritische Frequenzabstand die Resolution der Cochlea repräsentiert.

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## ACTIVE OTOSCLEROTIC FOCI IN THE STAPES

### *An Electron Microscopic Study*

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**Abstract** A total of 12 undecalcified and decalcified otosclerotic stapes containing active spongiotic lesions were examined using an electron microscope. Evidence of active osteolysis was seen in all specimens but osteoclasts were observed in only four. The advancing front of the spongiotic lesion was 'moth eaten' due to the mineralization of canaliculi and lacunae. The demineralizing process appeared to be initiated by degranulation of lysosomes by the degenerating osteocytes. As bone resorption continued, poorly mineralized new bone was laid down by osteoblasts which showed mitochondrial swelling. The possible implication of this mitochondrial swelling is discussed in relation to the pathology of the otosclerosis.

Considerable interest has been raised in recent years concerning the basic mechanisms involved in bone destruction and remodeling by otosclerotic foci (Rüedi, 1969; Chevance et al., 1969; Clarke, 1969). Chevance et al. (1970) proposed that the resorption of bone in the otosclerosis was mainly mediated by osteocytic osteolysis in which lysosomes containing acid hydrolases were released into the bone matrix. This process presumably led to the demineralization and remodeling of the ground substance that was controlled by the bone cells. These bone cells were inferred to be genetically normal in otosclerosis, and when new bone is laid down by these cells, they produced normal ground substances which either

failed to mineralize completely or developed sclerotic bone with an abnormal crystalline formation.

Many investigators attempted to study the demineralization and remineralization of the otosclerotic bone using the X-ray diffraction technique (Meurman et al., 1967; Puhakka, 1971), microradiogram (Clarke, 1964) and tetracycline labeling method (Rockert et al., 1965; Roberto et al., 1973). However, the information as to the cellular involvement leading to the pathological events was limited owing to the difficulty of preparing mineralized specimens for an ultrastructural investigation.

This study was undertaken in order to elucidate the fine structures of the bone cells and to shed light on the dynamics of mineralization in active otosclerotic foci in both undecalcified and decalcified otosclerotic stapes.

### MATERIALS AND METHODS

A total of 12 otosclerotic stapes with active otosclerotic lesions obtained from patients who underwent surgery was examined. Three percent cacodylate buffered glutaraldehyde (pH 7.2-7.4) was used as an overnight fixative, and the specimens were post-fixed in cold 1.3% osmium tetroxide buffered osmic acid (pH 7.2-7.4) for 1 hour. Six specimens were decalcified in 4.5% EDTA for 1-4 days and washed in Tyrode's

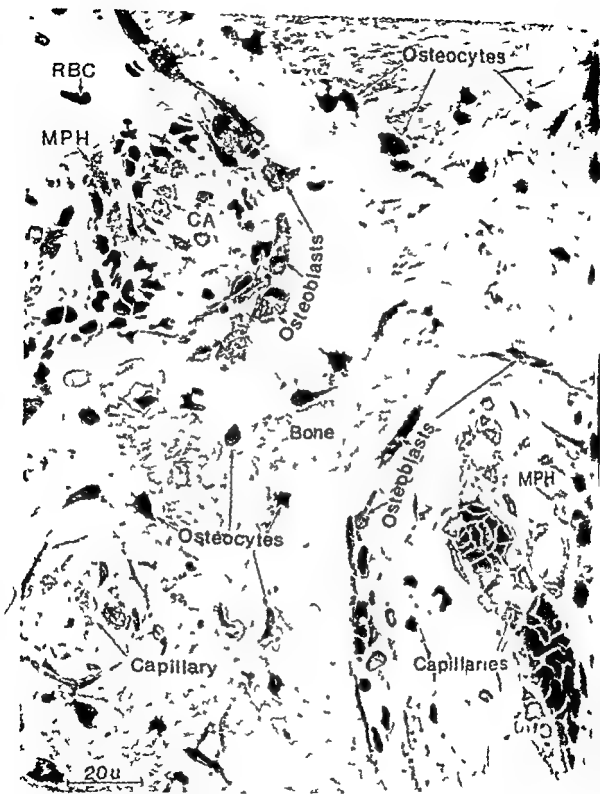
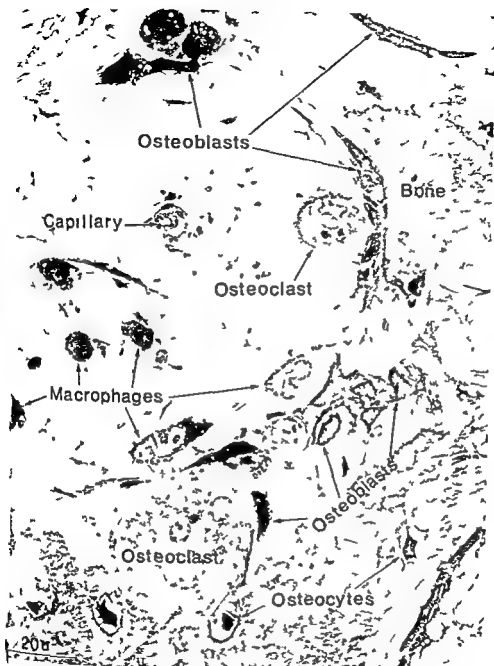


Fig 1 A low-power electron micrograph of an active otosclerotic lesion shows enlarged marrow spaces containing osteoblasts, macrophages (MPH), RBC and blood capillaries (CA). Numerous osteocytes occur in the lacunae. Decalcified.



2 An overall view of the active lesions shows numerous osteoblasts, macrophages and osteoclasts. Decalcified

tion for 1 hour before being embedded in ion as described earlier (Lim 1970) and the remaining 6 specimens were embedded in hard epoxy resin (Epon 812) without decalcification. The groups of specimens were thick sectioned

with a glass knife for phase-contrast microscopy and thin sectioned with a diamond knife for electron microscopy. Silver or gold colored sections were then placed on a carbon-coated formvar membrane and



Fig 3 (A) A demarcated mineralized bone (Bone) with primitive collagen (L) and active otosclerotic lesions (A). (B) An example of clear demarcation between mineralized bone and unmineralized collagen fibers in the advancing front of an active otosclerotic lesion. A dissolving lacuna (L) with

numerous dense granules (arrow) is shown. (C) An active lesion shows a demarcated appearance caused by demineralization along the canalicular. A degenerating osteocyte (Oc) in a proximal lacuna. Collagen fibers (C) are exposed in demineralized area. Arrows point to alveolar spaces. Undecalcified

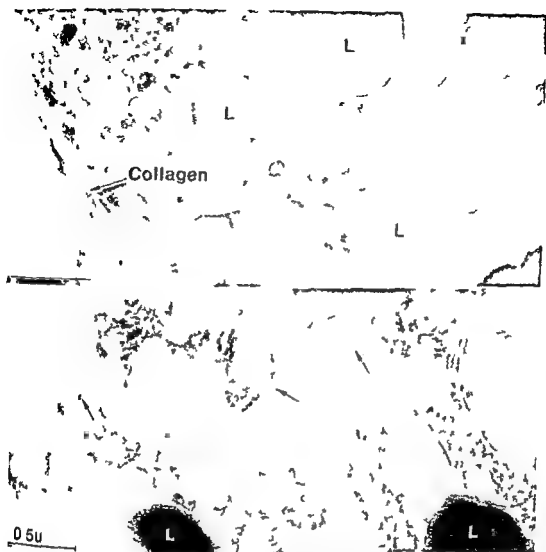


Fig. 1 (A) A resorbing lacuna of an active lesion contains numerous lysosomal granules presumably from degenerating osteocytes. Undecalcified (B) A close up view of the

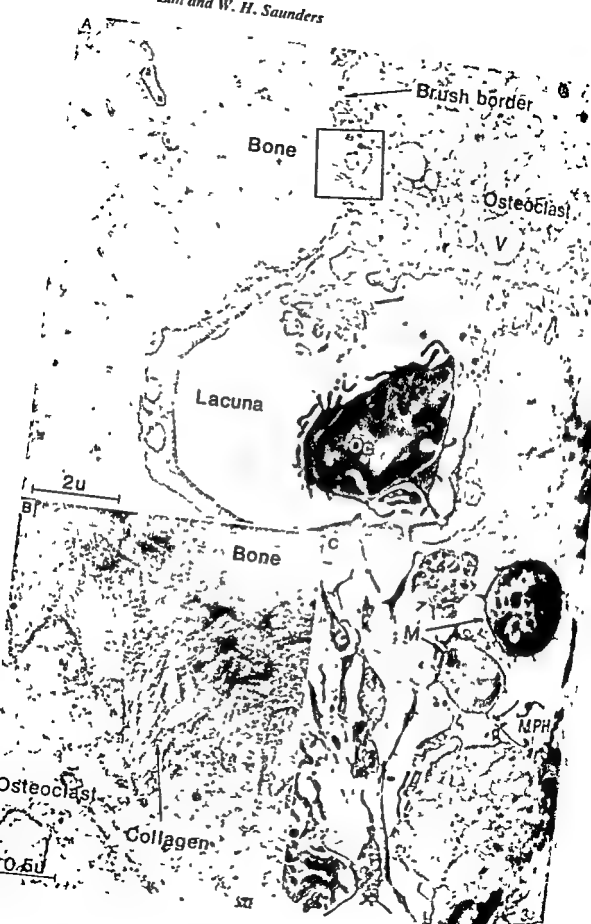
margin of a resorbing lacunar wall shows numerous apatite crystals (arrows). Dark bodies are degranulated lysosomes. Undecalcified.

## OBSERVATION

The stapedial bones with active otosclerotic lesions were spongy due to an extensive marrow infiltration and enlarged marrow

spaces filled with loose connective tissue. Both the bony resorption and new bone formation were evidenced by a large number of osteocytes, osteoclasts, macrophages and osteoblasts in the same specimen (Figs 1 and 2). Lacunae were occupied with osteocytes in the active lesions in contrast to the normal stapes where the lacunae were usually empty (Fig. 1).

When mineralized otosclerotic foci were examined, demineralized demarcation was clear



on an ultrastructural level in cases where bone appeared to be dense (Fig 3 A and B) In other cases, the advancing front showed the eaten appearance caused by the resorption of lacunar walls and canaliculi, and by the adic residual calcified islands of ground spaces among demineralized collagen fibers (Fig 3 C) In these resorbing lacunae, degenerating osteocytes and numerous annulated lysosomes were often seen in recent stages of the progression of degranulation and demineralization (Fig 4 A and B) In a magnification view, bone-apatite crystals were loosened at the demineralizing front out osteoclasts (Fig 4 A)

In addition to numerous degenerating osteocytes, osteoclasts were also observed in numbers in the area of bone resorption in 12 of the 12 spongy bones examined (Fig 4 B) In one case, an osteoclast was destroying bone tissue including an old lacuna (Fig 4 C)

The osteoclast, in general, was multi-nucleated and possessed a large cytoplasm which contained numerous mitochondria, ribosomes, vacuoles and Golgi apparatus. A microvilli like brush border made contact with the bony surface which showed evidence of demineralization with exposed bone collagen fibers (Fig 5 B) Crystals resembling bone apatite were observed between the borders of cytoplasmic processes in vacuoles of the osteoclast, indicating an active role of the osteoclast in the demineralization of bone.

Macrophages were also seen in large numbers in marrow spaces, particularly where active bone resorption was taking place. In

these cells, we observed numerous cytoplasmic inclusions such as lysosomal granules and lipid droplets and well-developed pinocytotic vesicles indicating active phagocytosis (Fig 5 C) In some instances, crystallites were found near macrophages in the marrow spaces, although it was not certain whether the crystallites were taken up by these cells. There were also numerous monocytes in the marrow space giving the impression that macrophages might have originated from them.

As the bone destruction continued, small capillaries were budding into the marrow spaces (Fig 2), giving the appearance that osteolysis preceded the capillary infiltration. In some areas adjacent to an active lesion, evidence of capillary degeneration was seen in the marrow space (Fig 6), where only scanty connective tissue and few cells were observed. Still, whether the capillary degeneration preceded the more active cell infiltration could not be determined. As the number and size of blood capillaries increased, the osteoblasts became more numerous, and they were lined up along the osteoid surface facing the marrow cavity (Figs 1 and 2). They showed a slender cell body with a dense cytoplasm due to its rich ribosome contents. Rough endoplasmic reticulum was extensively engorged, and Golgi's apparatus was well developed indicating active cell function. Numerous finger like cytoplasmic processes made contact with those of neighboring osteoblasts (Fig 7). The cytoplasmic processes were also embedded in the loose collagen layers immediately adjacent to the bone. These collagen fibers appeared to have been freshly laid down by the osteoblast and were not mineralized in these active otosclerotic regions. The osteoblasts that produced non mineralized collagen fibers showed severe mitochondrial swelling (Fig 6 A) similar to the swelling in the osteoblasts of experimentally induced atrophic rhinitis (Fetter & Capen, 1971). Precollagen fibrils were abundant in the area immediately adjacent to the osteoblasts, and they then appeared to reaggregate forming mature collagen fibers (Fig 7 B).

Fig 4 (A) A close-up view of an osteoclast actively engaged in bone resorption. Part of the lacuna is destroyed by the osteoclast. A small osteocyte (Oc) is seen in a large partially destroyed lacuna. V vacuole. The square is indicated in (B). Decalcified. (B) A high power view of a brush border reveals that collagen fibers of the bone are being resorbed by the osteoclast. Decalcified. (C) A macrophage (M) containing numerous lipofuscin and lysosomal granules is seen in the marrow space of an active lesion. At macrocytes. Decalcified.



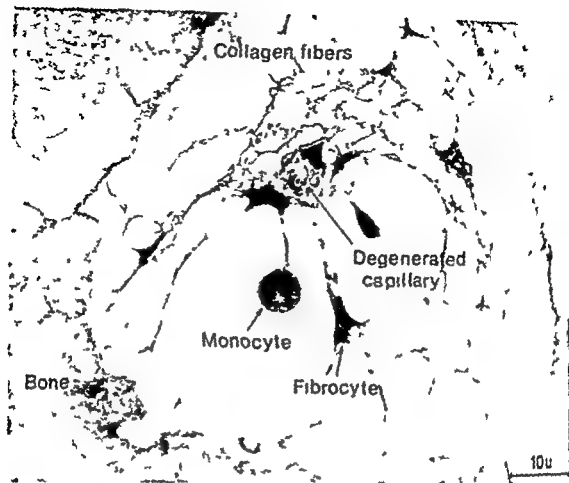
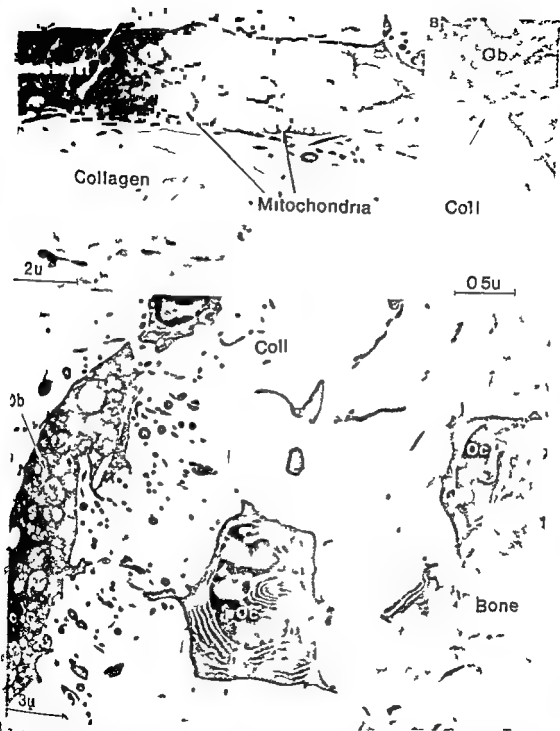


Fig 6 A marrow space closer to an active lesion shows a degenerating capillary (arrow) and a paucity of connective tissue and cells. Decalcified

As an osteoblast became completely isolated by newly laid down collagen fibers this isolated cell became a preosteocyte which still maintained the structural characteristics of the osteoblast such as a large cytoplasm-nuclear ratio, well-developed endoplasmic reticulum (ER) and numerous mitochondria (Figs 7C and 8A). In these preosteocytes lacunar walls were rough and surrounded by a large amount of unmineralized collagen fibers lacking the Rouget Newman zone (Fig 8A). When the lacunar wall was mineralized a typical Rouget Newman zone formed by dense unmineralized ground substance could be recognized (Fig 8B). The osteocytes occupying the lacunae in newly formed otosclerotic bone often possessed infolded nuclei and abundant ER. This appearance was in contrast to the

osteocytes found in demineralizing old bone. At the advancing front of the otosclerosis had large round nuclei and a small amount of cytoplasm with numerous lysosomal (Fig 8C).

Ground substances of newly formed otosclerotic bone were made mainly of collagen fibers and amorphous ground substance that became mineralized (Fig 8C). Fibers in the old lacunae undergoing resorption contained broad collagen fibrils (Fig 8D) as reported by Rejzdon & Sørensen. In the early stage of calcification there were numerous calcospherites which were found in association with nucleation centers similar to those found in the resorbing bone earlier (Fig 9A). When the otosclerosis was completely mineralized it showed



7 (A) A portion of an osteoblast actively engaged in collagen formation is shown. Numerous collagen fibers are seen nearby the osteoblast containing enlarged ER and swollen mitochondria. Decalcified (B) A higher power view of osteoblast bone junction shows

re-culin-like precollagen (arrow) and collagen fibrils (Coll). Decalcified (C) An electron micrograph depicts an osteoblast actively secreting collagen fibers (Coll) and a preosteocyte (pOc) that is being walled off and an osteocyte (Oc) in a partly mineralized lacuna. Decalcified

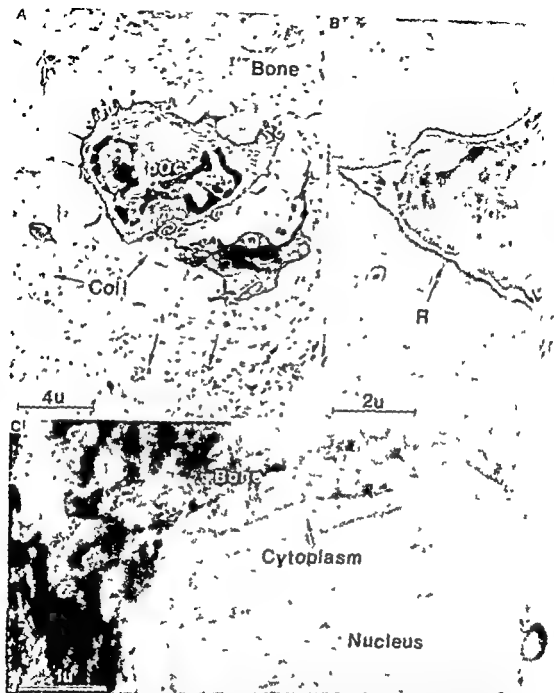


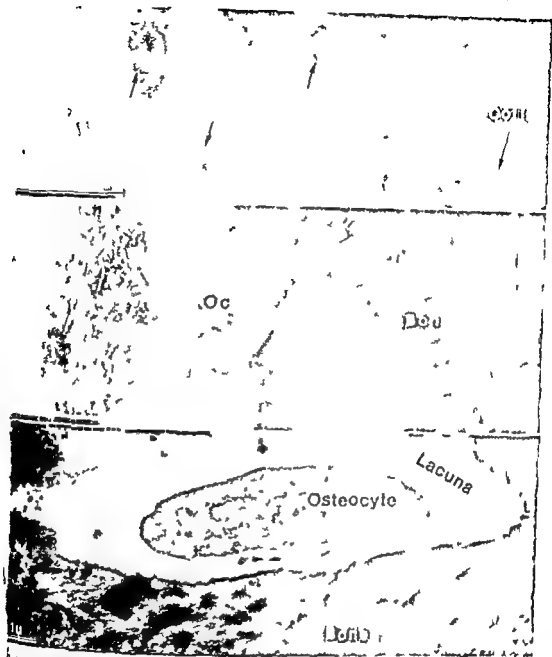
Fig. 8 (A) Preosteocytes (pOc) are seen in newly formed lacunae that are partly mineralized. Arrows point to nucleation centers of early mineralization. Coll: unmineralized collagen. Decalcified. (B) An osteocyte (Oc) is occupying lacuna with a distinct Rouget Newman zone (R). Bone is

well mineralized. Decalcified. (C) An osteocyte nucleus, scanty cytoplasm and dark granules occupies a well-mineralized lacuna in decalcified bone.

mely irregular patterns of crystal deposition due to the irregular collagen fiber arrangement which was in contrast to the normal bone where the fibers were organized in lamellar fashion (Fig. 9B and C).

#### DISCUSSION

There have been numerous hypotheses concerning the pathogenesis of the osteosclerosis. It is generally recognized that the development and progression of the osteosclerosis is



(A) Calcospherulites (calcospherulites) are indicated by arrows in an active otosclerotic lesion. Bone resorption appears to be closely related to the resorptive substance (arrows). Coll = unmineralized collagen. Undecalcified (B) A well mineralized otosclerotic

lesion shows in deep layers (crystal lines with a thin film) while the resorptive osteocyte (O) appears regenerated. Undecalcified (C) A osteocyte in a deep layer of stapes with well (resorptive) osteoclasts.

in several stages (1) destruction of the endosteal bone mediated by osteoclasts and osteoclasts (2) formation of immature basophilic bone and (3) repetition of the remodeling process of resorption and new bone formation

which eventually becomes highly mineralized and acidophilic having a mosaic like appearance (Schuknecht 1971). This concept of different stages is also supported by enzyme histochemical studies (Allert &

Covell 1961, Alberti & Tarkannen 1963). On the other hand Kelemen & Linthicum (1969) observed that every otosclerotic focus contained spongiotic and sclerotic portions and, therefore, these two portions did not represent young and old lesions. The present study did not cover the entire spectrum of the otosclerosis but it was concerned mainly with the pathology involved in an active spongiotic lesion.

It is generally accepted that the otosclerosis develops in predilected areas of the temporal bones and stapes. For frequent occurrences in certain families, the genetic factors in the development of this disease have been implicated. It is well recognized that genetic disorders of connective tissue can also be manifested in hypophosphatasia in which the bone lesion is caused by the inability of the osteoblasts to elaborate calcifiable matrix due to alkaline phosphatase deficiency and in osteogenesis imperfecta in which collagen is anomalous in its amino acid sequence (McLean & Urst 1968). Holdsworth et al (1973) suggested that the defective regulation of glycolysis and the resultant increase in aerobic metabolism by an aberrant phosphofructokinase (PFK) enzyme are the underlying causes of the otosclerosis. Recent electron microscopic studies also reported the presence of abnormal collagen fibers and bone minerals in otosclerotic bones (Reydon & Smith 1968, Meurman et al 1968). These abnormalities were inferred to be the result of malfunctioning bone cells which produced abnormal ground substances.

In our study, the newly formed collagen fibers that walled off new preosteocytes were not well mineralized. Whether the failure of mineralization was due to the peculiarity of the matrix formed by bone cells (Arslan & Ricci 1963) or due to the failure of bone cells to mobilize bone minerals could not be determined on the basis of a morphological study alone. However it appeared that the newly formed bone lacked dense ground substance in which bone minerals were ordinarily depos-

ited. There was no gross abnormal arrangement of collagen fibers per se in the lesion. We could not be certain whether the changes in the osteocytes in newly formed lesions represent pathological changes. They represent different physiological states.

Fetter & Capen (1971) reproduced active changes of osteoblasts and osteocytes in normal pig turbinate by inoculating turbinates obtained from nasal turbinates which spontaneously developed atrophy. Osteocytes in the experimental animals showed marked swelling of mitochondria which was not seen in normal animals. The swelling of mitochondria in osteoblasts in active otosclerosis observed in this report might be the reason for the malfunctioning of mitochondria since the mitochondria houses enzymes needed for energy metabolism and transport. We considered the possibility that the mitochondrial swelling was an artifact. However in view of the fact that cell organelles including mitochondria in bone cells in the same section were normal, we concluded that the mitochondrial swelling was not an artifact. Further cytochemical studies are required to determine whether mitochondria in these osteoblasts are malfunctioning.

Chevance et al (1970) recently suggested that osteocytic osteolysis was the resorbing mechanism in the initial lesion. They further suggested that lysosomal hydrolases released from lysosomes by the osteocyte caused demineralization of bone. These authors were able to see the advancing front of the otosclerotic lesion. They agreed with their findings that the evidence of degranulated lysosomes and disorganized cell organelles in the advancing front of the otosclerotic lesion appeared that enzymatic degradation of bone initiated by osteocytic osteolysis as suggested by Chevance was the main mechanism involved.

bone resorption in otosclerosis although osteoclasts were found in active lesions. Generalized microfoci, described by earlier investigators using decalcified tissue (Smith & Chevance et al, 1970), were found in the present study utilizing undecalcified tissue. These microfoci are related to the resorbing canaliculi in which chemical mediators such as acid phosphatases could be easily transported. The mechanism by which degranulation of lysosomes is accomplished in otosclerosis is unknown. It has been demonstrated that the thyroid hormone and vitamin D activate or inhibit lysosomal enzymes in bone cells. Besides these systemic factors, it has been shown that local factors can, in part, regulate the bone resorption and bone formation (McLean & Jorgensen, 1968). Conceivably, the local factor regulating bone resorption and bone formation, which are under genetic control, are malfunctioning in otosclerosis. It is also conceivable that the degeneration of capillaries in the marrow space, observed in the present study, in otosclerosis, as it is known that degeneration of the spiral vessels was the underlying basis of genetic cochlear degeneration in guinea pigs (Kikuchi & Hilding, 1967). Many investigators using light microscopy have already reported the presence of osteoclasts in an active otosclerotic lesion (Henner et al, 1960; Nager, 1969; Schuknecht, 1970). However, electron microscopic studies have failed to show osteoclasts in the surgically removed otosclerotic stapes (Reydon & Smith, 1970; Chevance et al, 1970; Lim, 1970; Nager, 1971). On the basis of their failure to find osteoclasts in the surgical specimens, Henner et al (1970) felt that osteoclasts had a secondary function in resorption of bone in otosclerosis. Nevertheless, Gonzales & Jorgensen (1961) confirmed the bone resorption function of the osteoclasts by showing bone resorption crystals (calcium phosphate) seen in the cytoplasmic processes and invaginated canaliculi of the osteoclast. The present findings are revealing that osteoclasts were actively

engaged in the removal of the bony matrix in certain active otosclerotic lesions, agreed with earlier light microscopic reports (Henner et al, 1960; Nager, 1969). Therefore, it could be concluded that both osteoclastic and osteolytic osteolysis might occur concurrently in certain active lesions. It was also apparent in the present study that macrophages were actively engaged in phagocytosis of dead cells and destroyed bones. Perhaps osteoclasts could help to dissolve bone minerals and collagen, but the waste products could be then taken up by the macrophages.

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Insgesamt zwölf entkalkte und entkalkte otosklerotische Stapes in aktiven otosklerotischen Herden wurden histomikroskopisch untersucht. In allen Fällen wurden deutliche Zeichen von osteolytischer Aktivität nachgewiesen. Osteoklasten hingen nur in vier Steigbügeln beobachtet. Die aktive Front der spongiosen Laesionen erstreckte sich auf die Kanaliculi und wurde von Motten zerfressen. Der Demineralisationsprozess scheint durch eine Degranulation der Lysosomen durch degenerierende Osteozyten eingeleitet zu werden. Während die Knochenresorption fortgesetzt wird, dürfte mineralisierter neuer Knochen durch Osteoblasten, die eine Schwellung ihrer Mitochondrien aufweisen, angebaut. Die mögliche Bedeutung dieser Schwellung der Mitochondrien für die Pathologie der Otosklerose wird diskutiert.

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## INCORPORATION OF RADIOACTIVE CALCIUM INTO OTOLITHIC MEMBRANES AND MIDDLE EAR OSSICLES OF THE GERBIL

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<sup>45</sup>CaCl<sub>2</sub> was injected into gerbils in single or multiple doses and the resulting radioactivity in serum, otolith  $\text{CaCO}_3$  bone samples and selected labyrinth epithelium was determined by liquid scintillation spectrometry. Incorporation into both utricular and saccular otoconia occurred at the rate of 0.06-0.07 nmole  $\text{Ca}^{++}$  per day corresponding to a fractional rate of uptake of approximately 11 days. The rate of incorporation of  $\text{Ca}^{++}$  for the middle ear ossicles was 5-7 times that for the utricle and was similar to that for the otic capsule and stapes bone. The level of  $\text{Ca}^{++}$  was higher in the epithelial regions of the utricular membranous wall than in the non-pigmented areas of the utricular and ampullary and in the stria vascularis.

and are loosely held together by an organic substance which has not yet been identified.

It is not known whether there is loss and renewal of otoconia in adult mammals. In both fixed and unfixed material the otoconia can very easily fall off the membrane. Schuknecht has suggested that in man utricular otoconia perhaps may become dislodged into the posterior ampulla and cause a form of disequilibrium that he has termed "cupulolithiasis" (Schuknecht 1962, 1969; Schuknecht & Ruby, 1973).

We have found (Johnsson, 1971; Johnsson & Hawkins, 1972) in the saccule from the cat both in vivo and post mortem a substantial number of loose otoconia below the otolithic membrane, accumulated along the inferior wall of the saccule. It is quite possible that these crystals represent otoconia dislodged from the otolithic membrane. Reduction in size and number of otoconia in the temporal bones from several aged human patients was also reported.

There are thus indications that loss of otoconia, and perhaps a decreased production of them with aging, may occur in man and other mammals. Yet there is no information in the literature concerning regrowth of otoconia in adult mammals. There is a continuous growth of many fish otoliths (Pannella, 1971)

Otoconia in man and other mammals consist of crystalline calcium carbonate in the form of calcite (Carlström, 1963; Carlström & Strom, 1955) unlike the mineral component of bones and teeth which is principally calcium hydroxyapatite as the complex salt carbonate-hydroxyapatite. Ross & Peacor (1975) have recently given a detailed description of the otoconia in the rat. Contrary to popular belief the otoconia are not, perhaps with the exception of relatively few crystals in the innermost layer, dislodged, in the true sense of the word, in the otolithic membrane (Johnsson & Hawkins, 1971). They form a separate crystalline layer

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Fig 1 Macula sacculi (top) and utricle (bottom) from gerbil dissected out from the vestibulum. Each neuroepithelium is covered by the otolithic membrane with III otoconia. Left ear  $\text{OsO}_4$ .

Dohlman (1971) has shown regeneration of the cupula in the pigeon after surgical ablation and Werner (1940) described regeneration of the membranous layer of the saccular superstructure in guinea pigs approximately three months after the original membranes had been dislodged by exposing the animals to strong centrifugal force. Werner's drawing (1940, p. 139) of the macula sacculi covered by the new otolithic membrane shows, however, no regeneration of otoconia. Belanger (1960) showed some incorporation of calcium into otoconia of very young rats and also found that isolated and fixed otoconia were able to exchange  $^{45}\text{Ca}$  in vitro with a solution in which they were immersed. Recently Veenhof (1969), using autoradiography, was unable to demonstrate  $^{45}\text{Ca}$  incorporation into the otoconia of adult mice.

The present study was designed to investigate the possible growth or neogenesis of

otoconia by measuring the uptake of  $^{45}\text{Ca}$ . A gerbil was selected because relatively small doses of tracer could be given in relation to body weight, thus achieving a high radioactivity in the serum. This is not the case for mice because most of the labelled calcium is taken up by bone. An attempt was also made to study the incorporation of calcium into the pigmented and non pigmented portions of the membranous wall of the utricle. The technique of tissue dissection and liquid scintillation spectrometry represents the most sensitive quantitative method for the measurement of  $^{45}\text{Ca}$  uptake.

## METHODS

Sexually mature male and female (*Meriones unguiculatus*) weighing 100–150 g were used in these studies. Approximately 0.5 ml saline containing 0.4 or 4  $\mu\text{Ci}$   $^{45}\text{Ca}$  per body weight was injected intraperitoneally. For serum analyses the animal was anesthetized with ether and about 0.5 ml blood was obtained from the orbit. The blood was allowed to retract overnight in the test tube and residual erythrocytes in the supernatant serum were removed by centrifugation. The serum samples were analyzed for calcium by the murexide-solphthalein complexone method (Gill 1967) and for  $^{45}\text{Ca}$  by liquid scintillation spectrometry.

Tissue samples were obtained by the dissection techniques described elsewhere (Hawkins & Johnsson 1975). The animals with their otolithic membranes are shown in Fig 1. Initial fixation of the inner ear was accomplished by gentle perfusion with buffered  $\text{OsO}_4$  into the widely opened round window. Small samples of bone (ca. 50  $\mu\text{g}$ ) from the midshaft of the femur were removed, broken off and carefully cleaned of marrow and periosteal soft tissue under the dissecting microscope. The temporal bones and small samples were immersed in the  $\text{OsO}_4$  solution for 24 hours and then partially dehydrated.

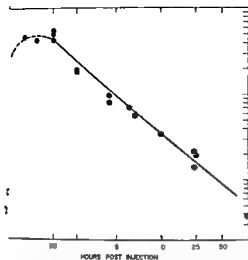


Fig. 2. Specific activity of serum  $^{45}\text{Ca}$  in adult gerbils of various ages after single injections ( $4 \mu\text{Ci } ^{45}\text{CaCl}_2$  per gram body weight intraperitoneally) plotted as a power function. The serum calcium concentration was assumed to be  $2.2 \text{ mM}$  for all animals.

ethanol. Samples of this solution displayed no measureable contaminating radioactivity. All specimens were transferred to 70% alcohol, which was changed several times during dissection. The fixation time was longer than usual because this procedure makes the otoconia more adherent to the membrane and surrounding crystals.

Nevertheless, several otoconia are often lost from the surface of the crystalline layer, particularly in the case of the saccule. With care it is possible to dissect out samples uncontaminated by bone dust. Several otoconial samples obtained from experimental animals, including gerbil, have been examined by powder X-ray diffraction. They have shown no traces of bone in the form of apatite.

Weights of otolithic membranes and bone samples were obtained after overnight drying on pre-weighed foil squares. The samples were then dissolved in HCl and the radioactivity measured.

Small pieces of utricular and ampullary membranous wall and stria vasculans from the lower basal turn were sampled as described below. The tissue samples were digested in hot  $0.25 \text{ M NaOH}$  and neutralized prior to scintillation counting.

## RESULTS

### Serum calcium

The concentration of calcium in the serum was determined from 4 animals to be  $2.21 \text{ mM} \pm 0.03$ . All further calculations are based on this value. After a single injection of  $4 \mu\text{Ci}$

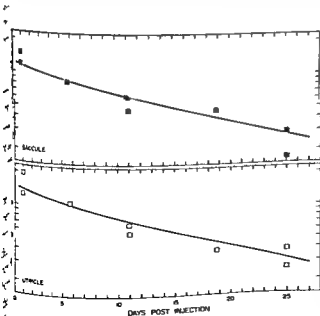


Fig. 3. Total cpm  $^{45}\text{Ca}$  for the paired otolithic membranes from each experimental animal plotted as exponential functions. The lines were fitted to the data points by the least squares method. Adult gerbils, males and females.  $^{45}\text{CaCl}_2$ ,  $4 \mu\text{Ci/g}$  body weight intraperitoneally.

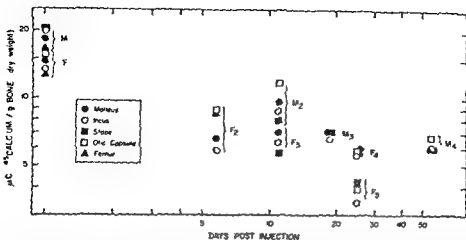


Fig 4 Selected determinations of  $^{45}\text{Ca}$  in bone samples from gerbils. The counts were obtained from whole middle ear ossicles and samples of comparable size taken from the bony ampullae and from femoral cortical bone.

All samples had been exposed to  $\text{OsO}_4$  and dried. M, males; F, females.  $^{45}\text{CaCl}_2$   $4\mu\text{Ci}$  intraperitoneally.

$^{45}\text{CaCl}_2/\text{g}$  body weight, the highest level of radioactivity in the serum is reached between 20–40 min (Fig. 2). There were no differences in the specific radioactivity of serum calcium between males and females.

#### Calcium turnover in otoconia and bone

Fig. 3 shows the retention of  $^{45}\text{Ca}$  in saccular and utricular otolithic membranes in eight animals which received single injections of isotope  $4\mu\text{Ci}/\text{g}$  body weight. Total counts are plotted rather than activity on a weight basis, since weight determinations proved to be imprecise. The lines were fitted to the data points using the least squares method, and best fit a straight line on a semilogarithmic plot. The half-life of calcium in utricular and saccular otoconia is approximately 11.3 days. Incorporation in an adult gerbil was compared to that in a six-week-old weanling after ten daily injections of  $^{45}\text{CaCl}_2$ . Based on the specific radioactivity of calcium in the serum, no difference was found in the uptake of calcium into otoconia in this experiment.

Incorporation and retention of  $^{45}\text{Ca}$  in middle ear ossicles was measured in the same animals and was similar to that in neighboring otic capsule and typical skeletal bone (Fig. 4). Higher values were found for all bones in 4 male gerbils ( $M_1$ – $M_4$ ) compared to five female

gerbils ( $F_1$ – $F_5$ ), whereas no such was found for the otoconia. In an experiment in which 40 daily doses of  $^{45}\text{CaCl}_2/\text{g}$  body weight were given, retention again was similar for the tibia and the other bone samples.

#### Uptake of calcium into the membranous walls

Uptake of  $^{45}\text{Ca}$  into different parts of the membranous walls was measured after single or multiple injections of  $^{45}\text{Ca}$  into the vestibular system. The pigmented part of the wall and portions of the wall which were completely non-pigmented were recognized under the dissecting microscope. The non-pigmented samples were taken from the anterior end of the utricle close to the oval or superior ampulla. Samples of the utricle were also taken to serve as controls, to cause the marginal cells of the utricle to be contaminated with pigment. The utricle was dissected loose from the spiral ligament, the lower basal turn of the cochlea was removed, and the utricle was cut out from the stria vascularis.

# **II $^{45}\text{Ca}$ uptake (cpm) into labyrinthine *elia***

els received  $4\ \mu\text{Ci } ^{45}\text{CaCl}_2/\text{g}$  body weight daily for the per of days indicated and were sacrificed one day he last injection except \*, sacrificed six days after jection

Experiment	Pigmented utricular wall	Non pigmented utricular wall	Ampullary wall	Stria vascularis
35	-	-	15	-
42	-	-	-	16
23	-	-	8	16
63	29	-	-	-
254	-	-	106	-

small iris scissors. The areas of the pieces estimated to be within 10% of each other. The highest incorporation was found in the pigmented samples (Table I). Those from stria vascularis and non pigmented areas of the saccular and ampullary walls contained measurable but significantly lower radioactivity.

## **Control experiment**

A control experiment was performed to insure that  $^{45}\text{Ca}$  in samples of otoconia and labyrinthine epithelium was not due to contamination by blood or body fluids. An animal was given  $2.5\ \mu\text{Ci } ^{45}\text{CaCl}_2/\text{g}$  body weight and sacrificed after 30 min when  $^{45}\text{Ca}$  in serum was at its highest level (Fig. 2). Radioactivity in serum was 1500 cpm/ $\mu\text{l}$ , but otolithic membranes, cupula and utricular epithelium had no activity. One of two samples of stria vascularis contained 17 cpm  $^{45}\text{Ca}$ .

## **DISCUSSION**

### **Calcium turnover in otoconia**

In contrast to the well-established morphology and composition of otoconia little is known about their origin and their turnover. Incorporation of calcium into these structures has been shown in young animals (3 to 14-day-old rats Belanger 1960, fetal to 2-month-old mice Veenhof 1969). The present

study clearly demonstrates incorporation of  $\text{Ca}^{++}$  into otoconia of adult as well as weanling gerbils. The rate of incorporation can be calculated from the radioactivity in the otolithic membrane one day after injection (Fig. 3) and the integral of the specific radioactivity in the serum over the same period (Fig. 2). This rate is 0.06 and 0.07 nmole calcium per day for the otoconia of the saccular and utricular membrane, respectively. The retention curves for calcium in the two types of membranes are almost identical (Fig. 3) and indicate a half life of otoconial calcium of approximately 11 days.

In order to compare the metabolic activity of the otoconia to that of the surrounding bone the weight of otoconial  $\text{CaCO}_3$  must be determined. Because of the small mass, measurements on a large pool of otolithic membranes would be required. On the basis of eight determinations each we estimated the weight of otoconial  $\text{CaCO}_3$  to be  $9\ \mu\text{g}$  per pair of saccular and  $17\ \mu\text{g}$  per pair of utricular membranes. This leads to a calculated fractional calcium uptake of 0.08% and 0.12% per day for saccular and utricular otoconia respectively. Thus calcium incorporation into these structures is 5-7 times slower than into the surrounding bone (see below).

### **Incorporation into bone**

It is evident that the uptake and retention characteristics are similar in the middle ear ossicles: the neighboring otic capsule bone and the femur (Fig. 4). The dry weight of the ossicles of the gerbil were determined to be: malleus  $1107\ \mu\text{g} \pm 55\ \text{S.D.}$  ( $n=18$ ), incus  $657\ \mu\text{g} \pm 53\ (18)$ , stapes  $126 \pm 18\ (10)$ . Assuming a calcium concentration of 22% in bones of all sources (Irving 1973, Vignen et al. 1973) the fractional incorporation rate of  $\text{Ca}^{++}$  into the various bone specimens was calculated (Figs. 2 and 4) to be 0.70% for a male ( $M_1$ ) and 0.47% for a female ( $F_1$ ), one day after a single injection of  $^{45}\text{CaCl}_2$ . The higher incorporation of calcium into bone of male gerbils may indicate a sex difference, but it should be

that the female gerbils were obtained from a different supplier.

The essentially identical rate of calcium uptake for all three middle ear ossicles is in apparent contrast to a study by Vigneri et al (1973). In the weanling rat these authors found the stapes to be much more active metabolically than the other ossicles. Differences in experimental conditions preclude direct comparison with the present results so that it remains unresolved whether this interesting metabolic variance is age or species dependent.

### Utricular 'dark cells'

On morphological grounds Lim (1973) has suggested that the specialized, osmophilic or electron-dense epithelium, termed 'dark cells', overlying melanocyte-containing subepithelial tissue in the vestibular labyrinth is responsible for removal of calcium from the otolithic system. Crystals dislodged from the otolithic membrane appear to lose their mineral content when attached to the dark cells. We have subsequently observed this relationship, in both light and electron microscopy, between otoconial crystals and the dark-cell epithelium in humans, as well as in guinea pigs and squirrel monkeys. Localization of  $^{45}\text{Ca}$  activity in pigmented areas and thus presumably in the dark cells can be observed in our experimental animals (Table I). Higher dose levels of  $^{45}\text{Ca}$ , together with quantitative control of amounts of tissue removed from different animals, might permit the determination of the time at which maximum activity appears in the dark cells and thus test the implication that they are responsible for the removal of otoconial  $\text{CaCO}_3$  from the endolymph. It should be noted, however, that no dark cell epithelium has been described in the mammalian sacculus. The fate of the saccular otoconia and the possible involvement of the specialized epithelium of the endolymphatic sac in their removal remains unclear.

In summary, this study has provided evidence for an active turnover of calcium in

otoconia, and has shown that in the adult bil calcium metabolism in middle ear is typical of bone in the skeletal system. In addition it has been demonstrated that dark cell epithelium contains higher levels of  $^{45}\text{Ca}$  than surrounding tissue suggests active participation of this tissue in calcium metabolism of otoconia. The biological interpretation of the uptake retention data for otoconia must remain at this point, however. The observed may reflect growth of existing otoconia, simple  $\text{Ca}^{++}$  between endolymph and  $\text{CaCO}_3$ , or a combination of these processes. Further investigations will be necessary to clarify these questions.

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Dr Roland Rouse of the University of Michigan Department of Geology performed the crystallographic. James W. Bruce and Vernon T. Maulbetsch co-artistic and photographic assistance. We thank P. Hawkins, Jr for advice and critical reading of the script.

### ZUSAMMENFASSUNG

Gerbils (*Meriones unguiculatus*) erhielten einmalige Injektionen von  $^{45}\text{CaCl}_2$ . Die relative Radioaktivität im Serum, im  $\text{CaCO}_3$  der Otolithen Knochen und Teilen der hautigen Wand des Innenohrs wurde im Szintillationszähler gemessen. Einbau in die Otolithen der Macula sacculi und utriculi mit einer Geschwindigkeit von 0.06–0.07  $\mu\text{Mol/L}$  pro Tag entsprechend einer prozentualen Einbaurete von 1%. Die Retention der Radioaktivität zeigte eine Halbwertszeit von etwa 11 Tagen. Die Einbaurete war 5–7 mal höher in den Gehörknöchelchen als in der Gehörknöchelchenwand. Die Einbaurete in den Ampullen und in den Ampullenkanälen war 1–2 mal höher als in den Ampullenkanälen.

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## INTERMEDIUS NERVE INVOLVEMENT AND TESTING IN ACOUSTIC NEUROMAS

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**Abstract** The clinical findings in 125 patients with surgically confirmed acoustic neuromas are presented with special regard to the involvement of the intermedius nerve in the diagnosis. In assessing the function of the intermedius nerve the examination of the nasolacrimal reflex and the sensation of taste on the anterior two-thirds of the tongue are used. The methods of investigation are described in detail. The material consisted of 20 medium sized and 105 large tumours, no intracanalicular tumor was found. Hearing loss was the initial symptom in 85% of the patients, 10% had tinnitus and 4% vertigo as the first symptom. Apart from the VIII cranial nerve symptoms a defective nasolacrimal reflex was the most significant evidence of cerebellopontine angle pathology. The test was positive in 65% of the medium sized tumours, in the entire material 85%. The figures are higher than the incidence of trigeminal nerve symptoms. This in contrast to the reports of most authors. The tests described are simple and quick to perform and it is emphasized that they should be applied to all patients with unilateral hearing loss of unknown origin.

Throughout the years a connexion between the size of the acoustic neuroma and the morbidity and mortality at operation has been clearly demonstrated. The larger the tumour, the greater the morbidity and mortality.

However, the early diagnosis, though much improved in the past 25 years, has by no means paralleled advances in the surgery of acoustic neuroma. The diagnosis of these tumours at an early stage while they are still small, is important, since total removal is possible in most cases, with very low mortality.

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and little or no damage to the most cases.

The purpose of this paper is: clinical findings in 125 patients with confirmed acoustic tumours with regard to the involvement of the intermedius in the diagnosis.

The motor fibres supplying the masticatory musculature form the main part of the facial nerve, but in a small portion made up of visceral afferent and general visceral efferent fibres. This minor part is called the intermediate sensory root of the facial nerve or the sensory root of the general visceral efferent fibres. It contains the lacrimal, the submaxillary and the sublingual glands as well as the glands of the oral cavity, and the parasympathetic (secretory) fibres of these structures. The taste fibres of the tongue pass centrally via the intermedius nerve. Cutaneous sensory branches of the facial nerve are the auricle and external auditory. In addition these regions are supplied by the sensory auricular ramus of the facial nerve (Brodal, 1969). In the internal acoustic nerve the intermedius nerve lies between the vestibular nerve above and the nervus intermedius below, hence the name.

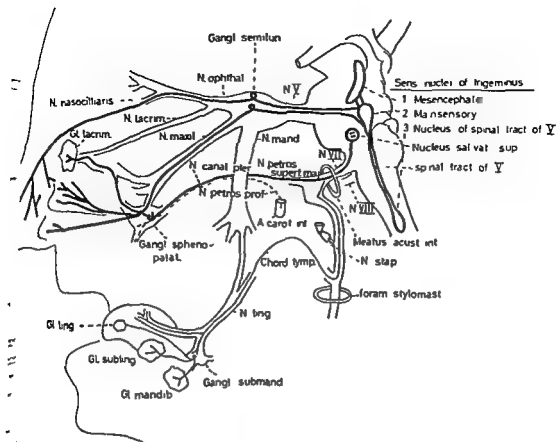


Diagram of the course of the nasolacrimal reflex (heavy lines)

assessing the function of the nervus intermedius we use the examination of the lacrimal reflex (NLR) and the taste on the anterior 2/3 of the tongue

The nasolacrimal reflex consists in lacrimation following chemical or mechanical stimulation of the nasal mucosa. The trigeminal nerve (maxillary and maxillary branches) form the afferent paths. Its efferent paths proceed from the superior salivary nucleus along the intermedius nerve, through the geniculate ganglion, the greater superficial nerve, through the sphenopalatine ganglion, the greater superficial nerve, through the nerve of the sphenoid canal (vidian nerve) to the sphenopalatine ganglion. From here they proceed through the sphenopalatine nerves to the maxillary nerve and via the zygomatic nerve to the lacrimal nerve and the lacrimal glands.

In 1900, Koster found that the secretomotor fibres to the lacrimal gland proceed along the above-mentioned course. However, Wernoe was the first to describe the whole reflex in 1929. That the reflex path takes this course has been proved in operated tumour cases by Zilsdorff in 1959. The taste sensation of the anterior two-thirds of the tongue is conveyed centrally through the gustatory fibres in the lingual nerve, the chorda tympani, the intermedius nerve to the nucleus tractus solitarius (Cushing, 1971).

## METHODS OF INVESTIGATION

To elicit the nasolacrimal reflex the following technique is used. One end of a piece of filter paper, 0.5 × 0.10 cm is inserted into the lower conjunctival fornix and folded twice at right angles over the ciliary border of the lower lid,



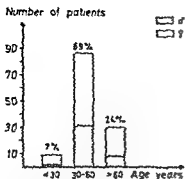


Fig. 2 Age distribution of 125 patients with acoustic neuromas

at the inferior lacrimal punctio. The paper is left in position while the examination takes place. For quantitative stimulation of the nasal mucosa the Elsberg olfactometer (Elsberg 1935, Zilstorff, 1959) is used, with benzine as the ingemmus stimulant. Benzine acts in most patients as a potent ingemmal stimulant and lacrimator. After the filter paper has been placed in the fornix a stream of saturated benzine fumes, 500 ml/min, is blown through one nostril directed at the olfactory region, for 30 sec while the patient breathes through the mouth. The paper is left in place for another 30 sec before it is removed. When the paper has dried, the lacrimation can be measured directly in millimeters. After an interval of at least 10 min the stimulation is repeated on the contralateral side. It is important to maintain this time interval, as the function may be reduced on the contralateral side for some time, due to overflow through the choana.

The lengths of the soaked tissue pieces on each side are compared measured in millimeters, a difference is considered significant if it exceeds 20% (Zilstorff 1959).

The sensation of taste is investigated by three different concentrations of the four basic qualities by the method of Bornstein (1940).

## MATERIAL

The material consists of 125 patients with acoustic neuromas, operated in the depart-

ment of neurosurgery, University of (Rigshospitalet) in the period 1957-1967. The same physician (Z) has performed otoneurological examination of all patients. An exact estimation of the tumour size is difficult and the findings are often different by otologists and neurosurgeons. However, we have followed the classification given by Pulec et al (1971). Small (intracanalicular) are confined to the auditory canal. Medium sized tumours extend into the cerebellopontine angle without V nerve involvement but without brainstem or cerebellar symptoms. Large tumours have indications of other nerve involvement, cerebellar or brainstem symptoms, and eventually increased intracranial pressure.

In the material, 20 medium-sized and 10 large tumours were found. The small tumours were not represented.

The age distribution is seen in Fig. 2. The majority are found in the 30-60 group—these tumours are rare below the age of 30. Women outnumbered men 1 to 1.

In Fig. 3, length of history is related to tumour size. Only 26% were examined in the first year and 38% had a history of more than 5 years. There is a tendency to longer history in the medium sized tumours but no definite connection between history and tumour size can be seen.

In Fig. 4 the patients' indications of the initial symptom are shown. In 85% hearing loss was the initial symptom, 10% indicated tinnitus and 4% vertigo as the first symptom.

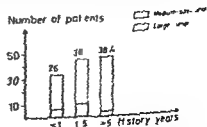


Fig. 3 Length of history in relation to tumour size in patients with acoustic neuromas

stents

HL = Hearing loss  
A = Taste test  
V = Vomiting  
D = Other

10% 4% 1%  
T V D Symptoms

First subjective symptom in 125 patients with  
neuromas

patient indicated reduced facial  
sensitivity initially

present in the entire material had normal  
hearing. Among the 20 medium-sized tumour  
patients 15 had only impaired hearing, while 5  
had loss in the ear involved. Among the  
large tumour patients considerably more had  
hearing loss (65%), while 35% had impaired hearing  
(Fig 6)

Of the patients with medium-sized  
tumours 15 had abolished differential - caloric  
even after irrigation with 20° water  
nystagmus (Fig 5), while this occurred in 84%  
of the patients with large tumours (Fig 6)

Of the patients with medium-sized  
tumours 15 had normal caloric function  
and no incidence of central spontaneous  
nystagmus and central positional nystagmus is  
seen in Figs 5 and 6. It should be noted that  
only one patient with medium-sized tumour  
had effective optokinetic nystagmus, while  
the patients with large tumours had  
effective and 8 show a comparison between the  
patients from the trigeminal and intermedius

The trigeminal function is estimated  
by the taste test and the nasolacrimal  
reflex examination as described above. It  
is clearly seen that in the medium sized  
tumours both taste and (especially) the NLR  
are considerably more frequently involved  
than the symptoms from the trigeminal nerve

(Fig 7). In the large tumours the pattern is the  
same (Fig 8), but the differences are less pro-  
nounced. It is evident, however, that loss of  
taste sensation and/or a defect NLR are found  
in the vast majority of the cases (85%) (Fig 8)

The motor function of the facial nerve and  
the sensibility of the external meatus have  
likewise been examined in the patients.  
Among the 20 medium-sized tumour patients,  
one had impaired motor function and impaired  
sensibility. We ascribe this to a previous zoster  
oticus in the involved ear. 23% of the pa-  
tients with large tumours had impaired motor  
function of the facial nerve, while only 6% had  
diminished sensibility in the posterior canal  
wall.

The material includes 7 bilateral tumours,  
and while neuromas as a whole are rare below  
the age of 30 (Fig 2), in our material only 7%,  
we found that 4 out of 7 patients with bilateral  
neuromas are less than 30 years old. All these  
4 young patients had signs of Reckling-  
hausen's disease, while the remaining 3 pa-  
tients had no signs of this disease.

In the material 6 patients had recurrence of a  
neuroma (5%).

## DISCUSSION

As mentioned earlier, it is of importance for an  
improvement of the postoperative morbidity  
and mortality to detect acoustic neuromas as  
early as possible or, more correctly, to find  
them when they are as small as possible. In

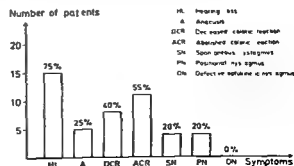


Fig 5 VIII nerve symptoms in 20 patients with medium-sized acoustic neuromas

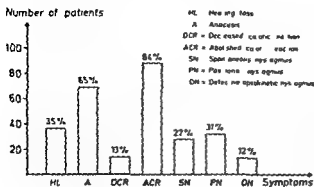


Fig 6 VIII nerve symptoms in 105 patients with large acoustic neuromas

our material none of the small, intracanalicular tumours were to be found, and only 20% of the tumours could be classified as medium sized. This distribution corresponds fairly well to the reports of other authors, e.g. Ozsahinoglu & Harrison (1974) and Ellis & Wright (1974). Some authors have been able to report materials with a considerable preponderance of small and medium-sized tumours, e.g. Fisch & Wegmüller (1974), House & Graham (1973), Montgomery (1973).

Regarding length of history, age and sex distribution, this material does not diverge from the reports of others (Cushing, 1917, Johnsen & Kristensen, 1949, Lundborg, 1952, Rembold & Tonnis, 1958, House, 1968, Fisch & Wegmüller, 1974, Ozsahinoglu & Harrison, 1974). With regard to the value of the examinations for the intermedius nerve involvement in the diagnosis of acoustic neuromas the opinions varies considerably. Fisch & Wegmüller (1974) do not mention these examinations. Sheehy (1968) states, that the examinations are routinely performed, but that they are of no differential diagnostic importance.

In our material we find that apart from the eight cranial nerve symptoms a defective nasolacrimal reflex is the most significant evidence of cerebellopontine angle pathology. Among the medium sized tumours the test was positive in 65%, in the entire material 85%. These figures are considerably higher than the incidence of trigeminal nerve symptoms, evaluated by facial and corneal sensitivity

The trigeminal nerve involvement was among the medium sized tumours and among the large. This stands in contrast to reports of most other authors (Cushing, Parker, 1928, Olsen & Horrax, 1944, Ellis & Paterson, 1951, Lundborg, 1952, Clarke & Garvey, 1953, Pool & Pava, 1957, Corne, 1969, Axelsson et al., 1973). However, these authors have not used the nasolacrimal reflex examination, as described by Zilstorff (1959).

Among the medium sized tumours taste sensation of the anterior two-third tongue is also more frequent than the trigeminal symptoms. The examinations of taste is performed *ad modum* Bornstein as a quantitative examination. This method, in our experience, proved to be more precise and easier to perform than electrogustometry (Krarup, 1959) in the clientele from neurological departments.

There are several methods testing function of the parasympathetic fibres in the intermedius nerve. Schurmer's (1903) test was an oculo-lacrimal test. Bobbin (1955) described a variation of this test for assessing decreased lacrimation, and the test used by Pulec & House (1964). Pulec (1971) has compared the Pulec & House method with that of Zilstorff (1959) and found that the quantitative measurement of Zilstorff is more sensitive and reliable.

Among the medium sized tumours only one patient with reduced motility

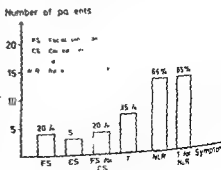
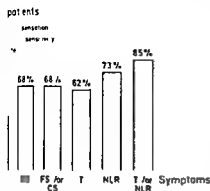


Fig 7 V and VII nerve findings in 20 patients with medium sized acoustic neuromas



VII nerve findings in 105 patients with large acoustic neuromas

and ascribed this to a previous zoster involved ear. Regarding the reason for at the sensory and parasympathetic facial nerve more frequently suffers from the involvement of the tumour we may only assume that the motor fibres are more resistant to the involvement than the small sensory fibres (Gasser 1929, Gasser, 1935, MacGladery 1951).

The reduced sensibility of the wall of the external meatus as described by Hitselberger & House (1966) and Rast & Feldmann (1973), we have been unable to find this symptom with any reasonable frequency. This may be due to the incomplete innervation of these regions, the vagus nerve, partly the intermedius nerve (Brodal, 1969).

It was reported in 1973 that a defective optokinetic nystagmus was a symptom with a frequency of 50% in large acoustic neuromas. We have not been able to confirm this, since we have only found a defective optokinetic nystagmus in 12% of the large tumours. In some cases adherence to or impression in the stem was found, but clinically this can only be confirmed in 12% (by presence of certain signs). It is however, not the same as in patients who have long tract signs and defective optokinetic nystagmus, since only 2 patients had both these symptoms. The brain stem is probably affected in a considerable time

before significant impairment of the optokinetic nystagmus occurs.

The early diagnosis of acoustic neuromas is of decisive importance for the course of the treatment. We believe that performance of NLR and taste examinations should be a routine part of the test battery used in the diagnosis of acoustic neuromas. They are simple and quick to perform, they do not demand any expensive instruments, and any otologist should perform them when dealing with unilateral hearing loss of unknown origin.

## ZUSAMMENFASSUNG

Die klinischen Befunde bei 125 Patienten mit operativ bestätigtem Akustikusneurinom werden vorgelegt und zwar mit besonderer Berücksichtigung der Einwirkung des Nervus intermedius. Um die Funktion des Nervus intermedius zu bewerten werden der Nasolacrimalreflex und die Geschmacksempfindlichkeit der vorderen zwei Drittel zur Zunge untersucht. Die Untersuchungsmethoden werden eingehend beschrieben. Das Krankengut besteht aus 125 Tumoren 20 mittlerer Grösse und 105 grossen intracanalicular Tumoren wurden nicht gefunden. Bei 85% der Patienten war das erste Symptom ein Hörverlust. Abgesehen von den Symptomen des 8. Hirnnervs war ein defekter Nasolacrimalreflex der bedeutendste Beweis einer Kleinhirnbrückenwinkelpathologie. Der Test war bei 85% der Fälle des gesamten Materials positiv. Diese Zahlen sind höher als das Vorkommen trigeminaler Nervensymptome. Dies widerspricht den meisten bisherigen Berichten.

Die beschriebenen Tests sind einfach und leicht anwendbar und es wird sehr empfohlen sie bei sämtlichen Patienten mit einseitigem Hörverlust unbekannter Ursache durchzuführen. Dadurch wurde die Anzahl grosser Tumoren (80% in unserem Krankengut) wesentlich reduziert.

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## BIOLOGICAL CONSIDERATIONS FOR THE USE OF HOMOGRAFT TYMPANIC MEMBRANES AND OSSICLES

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This report deals with some biological aspects of lation and preservation of tympanic membrane grafts. The structure and behaviour of membrane grafts depends largely on the reaction of the grafts with the tissue proteins. Due to these is no observable change in the microscopical of the membranes and ossicles was found up to 3 er alcohol and formaldehyde preservation. Pre in cialit showed a gradual disappearance of nuclei are as disintegration of the fibrous structures ive of the mode of preservation the fibrous tissue l to persist virtually unaffected for long periods ocular reaction found around the grafts pre r formaldehyde after heterotopic transplantation that tissue antigens are not completely destroyed on to transplantation in the middle ear is only ound fresh heterologous grafts and around the omologous grafts after previous sensitisation. As l in rats ossicles preserved in alcohol or cialit from revision operations in humans showed emodelling. Moreover in some cases these os nd especially the cartilaginous grafts showed areas ersion

e of homograft tympanic membrane and s in tympanoplasty has proved its value cent years. Although there might still be Mon as to whether superior results can erved with autograft fascia or ossicles, authors who have reported their ex ce with homografts agree that there are atable advantages in their use. The re- obtained are mostly founded on clinical ence, while basic knowledge is still grow- ) confirm their value and to adjust the omings of the techniques used. history of the use of homograft tympanic ranes is comparatively short. Chalat

(1964) is generally given the credit to be the first to have used vital homograft tympanic membranes for myringoplasty. Donor tympanic membranes were removed within 4 hours after death and kept in Ringer's solution for direct use. In 3 patients the grafts were placed over the de-epithelialised tympanic membrane of the acceptor. Two of the grafts failed to be incorporated, the third re-perforated later.

Only 2 years later, Marquet (1966) reported on the use of 17 preserved cadaver tympanic membranes and his experience was much more encouraging. The grafts were obtained within 12 hours of death, the epithelium was removed and the graft was stored in cialit (1:5000 solution) for about 4 weeks. In this first series of 17 patients, complete closure of the perforation was obtained in 15. In 1968 Marquet reported on 283 myringoplasties with a primary take rate of 81%, and another 14% take rate after revision. Improvement of knowledge and technique has raised the primary take rate to about 85%, which equals the results with temporalis fascia. MacKinnon (1972) describes the results of 112 tympanoplasties performed with homograft tympanic membranes preserved in 1:5000 solution of thiomersalate. The primary success rate was 82%. In a few cases he observed changes which could indicate incipient rejection. Histological sections of parts of the grafted tympanic membrane removed at a



2a



2b



2c

Fig 2 Micrograph of tympanic membranes preserved for 3 years in formaldehyde (a) (pars flaccida), alcohol (b) (pars flaccida) and cialit (c) (pars tensa). Note the disintegration of the fibrous tissue after cialit preservation. Only a few faint remnants of the nuclei can be seen in the fibrocartilaginous part of the annulus. H.E.  $\times 300$

studying the effect of the preservative. Various tissue components were preserved the same way. After preservation in cialit and formaldehyde the specimens were immediately decalcified in EDTA solution. After decalcification in EDTA solution in cialit the grafts were first preserved in formaldehyde 4% or alcohol for 24 hours and subsequently decalcified.

## RESULTS

### *Effects of Preservation on Structure of Graft*

Cialit, ethanol, and formaldehyde are preservatives which kill micro-organisms and render the autolytic enzymes inactive. In contrast to cialit, alcohol and formaldehyde do not preserve the molecular structure of the tissue profoundly. With alcohol being a non-fixative in the sense that none of its constituents joins itself to the proteins. Proteins are profoundly changed with coagulation. Formaldehyde is a cross-linker (reacts with protein) non-coagulant which hardens a protein gel without removing the water from the protein. Due to these properties preservation in alcohol involves shrinkage and hardening of the membrane. Formaldehyde also has a similar effect but shows less shrinkage. Preservation in cialit leaves the membrane very soft and pliable, and sometimes results in a spontaneous loosening of the epithelial layer from the fibrous layer within a few weeks. No decalcification was found in the ossicles after 3 years storage in the neutral formaldehyde solution. No essential difference can be observed between the microstructure of ossicles and tympanic membranes preserved in alcohol or formaldehyde. The hematoxylin-eosin stain. Twenty of the specimens were preserved for more than 3 years without any significant change.

Preservation in cialit during the staining process shows a loss of nearly all nuclear substance and slight disintegration of the fibrous structure. This is an important loss of stainability with

cal and histochemical staining methods (Figs 1 and 2)

### *Effect of Preservation on Behaviour of Graft*

#### *Orthotopic transplantation*

During the first week the tympanic membrane-malleus graft became surrounded by inflammatory cells, mesenchymal cells and capillaries. The graft was covered within 2 weeks by many layers of squamous epithelium on the outer side and 2 to 3 layers of mucosal cells on the medial surface. Thereafter the newly formed tympanic membrane gradually grows thinner. These phenomena are very similar to those described previously when other tissues were used for myringoplasty (Reijnen & Kuypers, 1971).

After 3 months the new membrane consists of a squamous epithelium consisting of 2 to 3 layers. The middle layer shows the fibrous tissue of the graft on both sides covered by newly formed fibrous tissue of varying density with small capillaries. On the medial side the membrane is covered by a thin mucosal layer. Similar observations were made 18 months after transplantation. In most cases the middle-layer of the graft appeared to be virtually intact, showing the characteristic fibre distribution with circular and radial fibres (Fig 3). After 18 months this characteristic distribution could not be observed in the newly formed fibrous tissue. The persistence of the donor fibrous tissue after longer survival times agrees very well with the observations made in humans (Marquet et al, 1973).

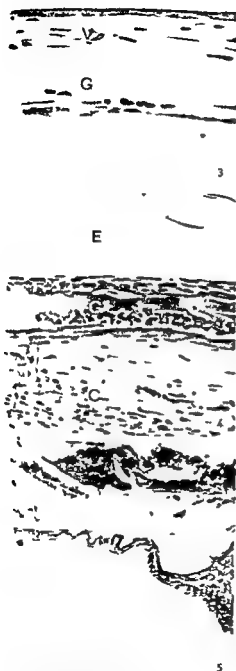


Fig 4 Tympanic membrane 10 months after myringoplasty with a formaldehyde preserved graft. The graft (G) is situated between a thin vital fibrous layer underneath the epithelium and a thick layer of loose connective tissue (C) containing round cells on the middle ear side. The presence of this latter tissue was observed only in those ears in which the paraffin support was left in position. External meatus (E). Heidenham  $\times 300$ .

Fig 5 Micrograph of a tympanic membrane (with annulus) 6 months after myringoplasty with an alcohol preserved graft. The new membrane mainly consists of loose connective tissue. Note the foldings in the graft possibly due to bad positioning. Heidenham  $\times 50$ .

Fig 5 Tympanic membrane 18 months after myringoplasty with an alcohol preserved homograft. The avital graft showing circular and radial fibres, is still present. Vital formed fibrous tissue (V), external meatus (E). H.E.



As in earlier observations, made with preserved incus grafts (van den Broek & Kuypers, 1974) the malleus attached to the tympanic membrane grafts and preserved in cialit or alcohol showed newly formed bone in some cases.

In the animals in which the paraffin support was left in the middle ear, the tympanic membrane showed a persistent infiltration of inflammatory cells throughout the observation period (Fig 4).

The final thickness of the new membrane appeared to be highly dependent on the size of the graft. Fig 5 shows a specimen in which the thickness of the membrane seems to be determined by the foldings in the graft. No essential difference has been observed between the behaviour of the tympanic membrane grafts preserved in alcohol, formaldehyde or cialit.

#### *Heterotopic transplantation*

The course of the reaction after heterotopic transplantation of preserved tympanic membrane-malleus grafts is very similar to that observed with preserved incudes (van den Broek & Kuypers, 1974). After preservation in alcohol or cialit, new bone formation was observed in most cases in the malleus, but not after preservation in formaldehyde. Apart from this, formaldehyde preserved grafts appeared to be surrounded by mononuclear cells throughout the observation period, in contrast to those preserved in cialit or alcohol (Fig 6). A most remarkable finding was the extremely high resistance of the fibro-cartilaginous part of the annulus and the fibrous content of the grafted membrane to resorptive activity of the host (Fig 7). Even after 18 months the fibrous tissue appeared to be nearly unaffected, irrespective of the mode of preservation, while large areas of the malleus bone were resorbed.

#### *Fresh incus grafts after sensitisation*

In contrast to the observations made on a large series of orthotopically transplanted fresh homologous incudes, which failed to show



Fig 6 Micrograph of a tympanic membrane formaldehyde 40 weeks after transplantation muscle. The graft is surrounded by mononuclear cells. H.E.  $\times 300$ .

Fig 7 Fibrocartilage of the annular part of the membrane preserved in cialit 18 months after transplantation into the leg muscle. There is no reaction to the graft and the structure is virtually unaffected.

clear phenomena suggestive of a reaction, resorption of fresh incus was observed in 3 out of 11 incudes previous sensitisation. This reaction was characterised by the presence of a large number of mononuclear cells around the graft and by resorptive activity, with or without the presence of giant cells (Fig 8). No preserved incus was tested in this series.



Fig 8 Fresh homologous incus graft 4 weeks after orthotopic transplantation in the middle ear of a previously sensitized animal. The long process of the incus revealing a large area of bone resorption is surrounded and invaded by mononuclear cells. Note the large giant cell. H.E.  $\times 90$ .

#### Homologous grafts

cases in which fresh heterologous incudes transplanted orthotopically or heterotopically a persistent massive reaction of mononuclear cells around the grafts was present during the observation period up to 12 weeks. Massive resorption of the transplanted incus was a normal finding at both sites (9 and 10).



#### Incus specimens

Incus grafts obtained from revision operations showed a great variety in histological structure. Nearly all homologous ossicular grafts preserved in toto showed areas of new bone formation. In these ears which underwent revision operation because of cholesteatoma or chronic otitis, large areas of the grafted incus sometimes appeared to be resorbed or replaced by fibrous tissue.

Homologous preserved ossicles obtained from non-inflamed ears which underwent revision because of dysfunction of the reconstructed ossicular chain in many cases revealed new formed bone to a varying extent. In some cases the graft was entirely dead. A most remarkable finding was that even in these non-



Fig 9 Fresh heterologous incus graft (rabbit) 4 weeks after transplantation into the rat middle ear. The graft shows large areas of resorption and is encapsulated in fibrous tissue with many mononuclear cells. Stapes (S). H.E.  $\times 90$ .



Fig 10 Fresh heterologous incus graft (rabbit) 5 months after transplantation into the rat leg muscle. Extensive monocellular reaction and large areas of resorption. H.E.  $\times 90$



Fig 11 Incus graft 11 months after transplantation. Graft removed because of dysfunction. Note small areas of resorption (arrows) and inflammation. H.E.

inflamed cases some of the homografts still showed clear areas of bone erosion (Fig 11).

In one case a large part of the graft was replaced by fibrous tissue. Apart from bone erosion was also observed in one graft (mainly consisting of dead bone 6 months after repositioning). Comparable resorptive activity was observed in homologous cartilage grafts (preserved in alcohol) used for an ossicular reconstruction (Fig 12) and repositioning because of poor functional results. In all cases signs of inflammatory were absent.

## DISCUSSION

The results of the experiments presented in this study deal with several aspects of the homografts. The first aspect which has been dealt with is the influence of several fixation modalities on the graft. The results presented show an important difference between the effect of preservation in alcohol versus formaldehyde versus preservation in ice. The physical properties and the microstructure of the tympanic membrane effects are due mainly to the different ways these preservatives react with the lipoproteins of the graft, especially the protein



*Fig 12* Part of a cartilage graft preserved in alcohol, removed from the middle ear of a patient because of dysfunction after 18 months. The cartilage is invaded and partially replaced by fibrous tissue. H.E.,  $\times 90$

The resulting "fixation" involves the histological structure of tympanic membrane and ossicles after preservation in alcohol or formaldehyde does not change with time. Even after 3 years the structure was unchanged. Because alcohol does not interfere to any large extent with the solubility of tissue compounds, fixation in an aqueous solution involves a partial loss of many soluble substances. This is different from a general loss of stainability, a disappearance of the nuclear substance of the cells, and a diminution of the mutual coherence of the various tissue elements, as is clearly visible in Fig 2. As a result of these effects, no observable difference could be established between the behaviour of orthotopically transplanted tympanic membrane grafts preserved in various ways. Neither the microscopic changes in the graft nor the host reaction around the graft were influenced by the type of preservation. The degeneration process of the tympanic membrane was similar to that observed when fresh autogenous tissues are used (Reynen & Kuipers, 1974) except for a very long persistence of the collagenous fibres of the tympanic membrane. These appeared scarcely affected 1 1/2

years after transplantation (Fig 3). Grafts of fresh fascia or fat are usually absorbed or unrecognisable after the same period of time.

These observations agree very well with the observations of MacKinnon (1972) and the case report of Marquet et al (1973) in which the persistence of the fibrous tissue of a cialit preserved tympanic membrane graft was demonstrated after 26 months.

The tympanic membranes transplanted heterotopically into leg muscle also showed a marked resistance of the fibrous tissue against resorptive activity, irrespective of the method of preservation. However, the persisting accumulation of mononuclear cells around the tympanic membranes preserved in formaldehyde is highly suggestive of a reaction to transplantation which can be explained by the fact that preservation in formaldehyde does not affect the transplantation antigens profoundly. Similar observations were made previously around formaldehyde-preserved incus grafts transplanted in muscle. A possible role of formaldehyde as a non specific stimulus causing this reaction was excluded (van den Broek & Kuipers, 1974).

The behaviour of the malleus as part of the tympanic membrane/malleus graft does not dif-



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## ERA-KENNLINIEN BEI NORMALHÖRENDEN PERSONEN

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**Abstract:** Bei 28 normalhörenden Jugendlichen (20-25 Jahre) wurden die ERA Kennlinien und die entsprechenden Latenzverläufe bei den Frequenzen 500 Hz, 1000 Hz und 4000 Hz gemessen und in der Form  $A/\mu V$  über HL/dB dargestellt. Zur Untersuchung der Altersabhängigkeit der AEP wurde bei zwei weiteren Gruppen (30-50 Jahre und 55-70 Jahre) die 1000 Hz Kennlinie ermittelt. Für den Bereich 10-50 dB werden die Gleichungen der Regressionsgeraden angegeben. Bei der Berechnung der Varianzen der Amplituden zeigte sich, daß die intraindividuelle Varianz signifikant unter der interindividuellen Varianz liegt. Prozentual als Varianzkoeffizient ausgedrückt, werden beide mit steigender Reizstärke geringer. Für die Latenzverläufe werden die nach der Methode von Verhagen angepaßten Exponentialfunktionen angegeben. Im Gegensatz zum Verhalten der Amplituden ergab sich bei den Latenzen keine signifikante Differenz der intra- und interindividuellen Varianzkoeffizienten. Auch zwischen den Varianzkoeffizienten der N1- und P2-Latenzen ist kein signifikanter Unterschied nachweisbar. Bei mittleren Reizstärken weist die Variabilität der Latenzen ein deutliches Minimum auf. Eine Altersabhängigkeit der AEP im Bereich 20-70 Jahre ist nicht festzustellen.

Für die Einschätzung der Ergebnisse von Messungen mit der Evoked Response Audiometry (ERA) im überschweligen Bereich ist es notwendig, die Amplituden Kennlinien und Latenzverläufe der evozierten Potentiale Normalhörender mit den entsprechenden Streubreiten vorliegen zu haben.

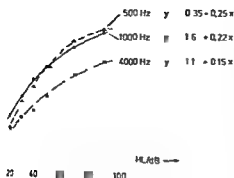
Obwohl solche Kennlinien bereits von der Arbeitsgruppe um Stange (Müller & Stange, 1971; Benning et al., 1972; Stange, 1973) veröffentlicht wurden, haben wir diese Messungen nochmals durchgeführt, da durch die

von den genannten Autoren gewählte Darstellung der Amplituden als „kortikale Erregung“ (die AEP-Amplitude wird aus einem reizunbeeinflussten Abschnitt gemittelten EEG berechnet, dividiert und nach Logarithmierung als Maß für die „kortikale Erregung“ angegeben) (Spreng & Keidel, 1967) die Übereinstimmung dieser Normwerte sehr erschwert wird.

### METHODIK

Als Versuchspersonen (Vp) dienten insgesamt 28 normalhörende Jugendliche im Alter von 20-25 Jahren sowie zwei Altersgruppen (30-50 Jahre und 55-70 Jahre) zu je 20 Vp. Die Ermittlung der Kennlinien bei 500 Hz, 1000 Hz und 4000 Hz erfolgte an einer Gruppe von 15 Jugendlichen. Jeder Vp wurden pro Frequenz 4 Kennlinienmessungen an verschiedenen Tagen durchgeführt. Zur Ermittlung der intraindividuellen Variabilität wurde bei 13 Jugendlichen der Kennlinienverlauf bei 1000 Hz je 11 verschiedenen Tagen gemessen. Für die Bestimmung der interindividuellen Varianz dienten die Messungen von allen 28 Jugendlichen Vp. Bei den Altersgruppen 30-50 Jahre und 55-70 Jahre wurde zur Ermittlung der Altersabhängigkeit nur die 1000 Hz Kennlinie gemessen.

Die Dauer des Tonreizes war 500 ms, die Anstiegs- und Abfallzeit (die A-



ERA Kennlinien für 500 Hz, 1000 Hz und 4000 Hz, gemittelt über die individuellen Mittelwerte von 17 20-25-jährigen Erwachsenen. Die Regressionsgleichungen gelten für den Bereich 10-50 dB.

in den 500 ms enthalten). Die Pausen zwischen den Reizen war 2 s. Das Audiometer (PAR 140) ist nach ISO Normiert. Mit diesem Audiometer wurde vor den Messungen auch die subjektive Hörschwelle der Vp ermittelt. Bei allen Vp war die Schwellenreicherung der individuellen Hörschwelle bei den 3 Frequenzen von der ISO Norm kleiner als 5 dB. Die Mitteilung wurde mit einem Averager (DL 102 A, ALAB) durchgeführt, der eine automatische Unterdrückung des Artefakts ermöglicht. Jeweils 64 Einzelantworten wurden gemittelt. Der EEG Verstärker (PAR 113) hatte einen Eingangswiderstand von 100 M $\Omega$ , die Grenzfrequenz war 30 Hz, die untere

Grenzfrequenz 0,3 Hz. Die durch diese Bandbegrenzung bewirkte Verzögerung des Signals liegt nach unseren Messungen bei 2 ms. Da die meisten Autoren mit ähnlichen Bandbegrenzungen arbeiten und die Verzögerung gering ist, haben wir die gemessenen Latenzen nicht mit diesem Wert korrigiert. Eine Beeinflussung der AEP-Amplitude ist bei den gewählten Grenzfrequenzen noch nicht nachweisbar. Die Ableitung erfolgte mit der standardisierten Elektrodenlage: Vertex gegen Mastoid des unbeschalteten Ohres und Erdung der Stirn. Die Vp lasen während der Ableitung Texte nach eigener Wahl. Als Amplitude des AEP wird die N1-P2 Differenz bezeichnet. Alle statistischen Berechnungen wurden auf einem LINC-8 durchgeführt.

## ERGEBNISSE

Abb 1 zeigt die ermittelten Kennlinien bei den Frequenzen 500 Hz, 1000 Hz und 4000 Hz. Die Kennlinien bei 500 Hz und 1000 Hz verlaufen praktisch gleich, bei 4000 Hz sind die Amplituden des AEP jedoch wesentlich geringer. Die in Abb 1 angegebenen Regressionsgleichungen wurden für den Bereich 10 dB bis 50 dB berechnet.

Zur besseren Übersichtlichkeit wurden die Standardabweichungen der Amplituden in Abhängigkeit von der Reizstärke gesondert in Abb 2 dargestellt.

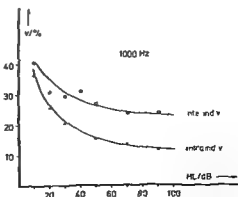
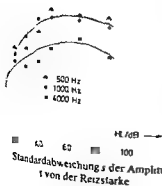


Abb 3: Intra- und interindividuelle Variationskoeffizienten  $v$  der AEP-Amplituden in Abhängigkeit von der Reizstärke ( $v = (s/M \cdot 100\%)$ ).



Standardabweichung  $s$  der Amplituden aus Abb 1 in Abhängigkeit von der Reizstärke.



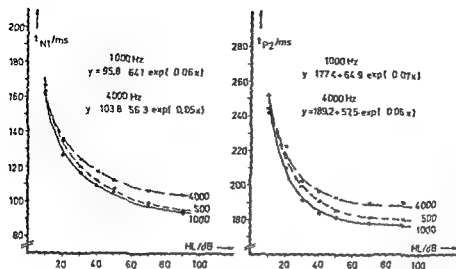


Abb 4 N1 und P2 Latenzen für 1000 und 4000 Hz bei 15 normalhörenden

Für 1000 Hz wurden die intraindividuelle und die interindividuelle Standardabweichung getrennt ermittelt und in Abb 3 als Variationskoeffizient  $v = (s/M) \cdot 100\%$  ( $s$  = Standardabweichung,  $M$  = Mittelwert) in Abhängigkeit von der Reizintensität dargestellt. Der intraindividuelle Variationskoeffizient ist der Mittelwert der bei 13 Vp aus jeweils 10 Potentialen pro Reizintensität berechneten individuellen Variationskoeffizienten. Die interindividuellen Variationskoeffizienten wurden aus den Abweichungen der individuellen Mittelwerte vom Gesamtmittelwert bestimmt. Im gesamten Intensitätsbereich liegt die inter-

individuelle Variabilität der Amplituden signifikant über der intraindividuellen.

Die zu den AEP Amplituden dazugehörigen Latenzen zeigt Abb 4. Sowohl für N1 als auch P2 liegt die Latenzkurve im Bereich 20–90 dB signifikant über der 1000 Hz Kurve (1% Niveau, darunter 5% Niveau) Differenzen zwischen 500 Hz und 1000 Hz sind nicht signifikant (Überprüfung mit t-Test). Für die 1000 Hz und 4000 Hz Kurven sind in der Abb 4 die Methoden von Verhagen (Rasch & Berger, 1967) angepaßten Regressionskurven angegeben. Die Standardabweichungen der Latenzen sind ebenfalls gesondert dargestellt.

Wie für die Amplituden so wurden auch die Latenzen bei 1000 Hz die intraindividuellen Variationskoeffizienten in Abhängigkeit von der Reizstärke dargestellt. Abb 6 zeigt diese Variationskoeffizienten der Latenzen von N1 und P2. Im Vergleich zu den Amplituden sind jedoch bei den Latenzen keine Differenzen zwischen intraindividuellem Variabilität nach Altersgruppen. Abb 7 enthält die 1000 Hz Kennwerte für 3 Altersgruppen 20–25 Jahre, 30–50 Jahre, 55–70 Jahre. Die Differenzen der Kennwerte sind nicht signifikant.

<sup>1</sup> Damit berechnet sich der intraindividuelle Variationskoeffizient zu:

$$v_{\text{intra}} = \frac{1}{k} \sum_{i=1}^k v_i$$

$$\text{mit } v_i = \frac{s_i}{\bar{x}_i} \cdot 100\%, \quad \bar{x}_i = \frac{1}{n} \sum_{j=1}^n x_{ij}$$

$$s_i^2 = \frac{1}{n-1} \sum_{j=1}^n (x_{ij} - \bar{x}_i)^2$$

Den interindividuellen Variationskoeffizienten erhält man aus der Formel:

$$v_{\text{inter}} = \frac{s}{\bar{x}} \cdot 100\%$$

$$\text{mit } \bar{x} = \frac{1}{n} \sum_{i=1}^k \sum_{j=1}^n x_{ij} \quad \text{und} \quad s^2 = \frac{1}{k-1} \sum_{i=1}^k (\bar{x}_i - \bar{x})^2$$

In unserem Fall gilt stets  $k = 13$ ,  $n = 10$

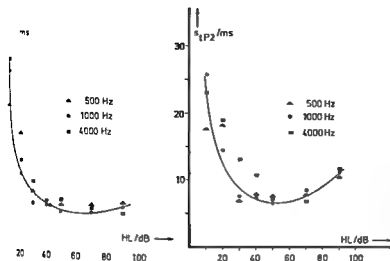


Abb 5 Standardabweichung  
s der Latenzen von Abb 4  
in Abhängigkeit von der Reiz-  
stärke

## DISKUSSION

nlmten

die Kennlinien wird im Gegensatz zu  
eren Autoren (Spreng & Keidel, 1967,  
Ber & Stange, 1971, Benning et al, 1972)  
direkte Darstellung der Amplituden in  $\mu V$   
r der Reizintensität in dB bevorzugt. Die  
rtstellung der AEP Amplituden als kortikale  
rgung wie sie von Spreng & Keidel (1967)  
geschlagen wurde, hat neben Vorteilen  
rminderung der Streuung) auch Nachteile.  
Bezugsgröße für die Berechnung der korti-  
m Erregung die aus einem reizfreien  
schnitt ermittelt wird, ist abhängig vom  
quenzgang des Verstärkers, wodurch die  
rgleichbarkeit der Ergebnisse erschwert  
nd ihre Berechnung von Hand ist zeit-  
fönd und fehleranfällig. Die maschinelle  
rechnung erfordert einen frei-program-  
erbaren Rechner. Da die Reizstärke  
hängigkeit der AEP Amplituden offen-  
ch nicht einer Potenzfunktion gehorcht,  
rft der Verlauf der kortikalen Erregung  
ch keine Gerade über den interessierenden  
sensitätsbereich 0-90 dB. Andererseits ist  
folge der komplexen zentralen Verarbeitung  
ch kein linearer Zusammenhang zwischen  
r Reizstärke in dB und der AEP Amplitude  
/ $\mu V$  zu erwarten. Da jedoch für den prak-  
tischen Gebrauch ein lineares Modell Vorteile

besitzt, wurde mit Hilfe eines statistischen  
Linearitätstestes nach Draper & Smith (1967)  
getestet, ob zumindest in dem Bereich 10 dB  
bis 50 dB eine lineare Abhängigkeit der Ampli-  
tuden von der Reizstärke in dB anzunehmen  
oder abzulehnen ist. Voraussetzung für die  
Anwendung dieses Testes ist das Vorliegen  
mehrerer Meßwerte pro Abszissenwert.

Die Anwendung des Testes sowohl auf die  
Mittelwertskurven bei den 3 Frequenzen als  
auch auf die individuellen 1000 Hz Kennlinien  
der 15 Vp ergab nur für 1 Vp Ablehnung

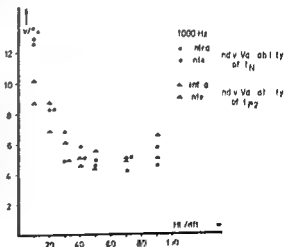


Abb 6 Intra und interindividuelle Variabilität  
n1 und n2 Latenzen in Abh. d. Reiz-  
stärke

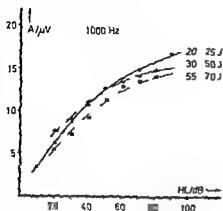


Abb. 7 1000 Hz Kennlinien der Altersgruppen 20-25 Jahre, 30-50 Jahre und 55-70 Jahre

des linearen Modells, für die anderen 14 individuellen Kennlinien sowie für die Mittelwertkurven der 3 Frequenzen wird im Bereich 10-50 dB ein linearer Verlauf nicht abgelehnt.

Um die Darstellung als kortikale Erregung zu simulieren, wurden alle Amplitudenwerte, die zur Berechnung der Mittelwertkurven dienen, logarithmiert und wieder getestet. Bei allen 3 Frequenzen ergab sich, daß ein linearer Zusammenhang zwischen Reizintensität in dB und der kortikalen Erregung in dB auch in dem engen Bereich 10-50 dB abgelehnt werden muß. Das ist als weiterer Nachteil der Darstellung der AEP-Amplituden als kortikale Erregung zu werten.

#### Frequenzabhängigkeit der Amplituden

Der Anstieg der Regressionsgeraden für 4000 Hz ist erwartungsgemäß deutlich geringer als bei den tieferen Frequenzen. Auf Grund ihrer Messungen stellten Davis et al. (1968) die Hypothese auf, daß bei gleicher Lautstärke auch bei unterschiedlichen Reizfrequenzen annähernd gleiche Antwortamplituden resultieren. Die geringeren Amplituden bei höheren Frequenzen, verglichen mit 1000 Hz, waren dann mit einer geringeren Lautstärke zu erklären. Da die Kurven gleicher Lautstärke jedoch zwischen 1000 Hz und 6000 Hz annähernd parallel verlaufen (ISO/R 131-1959), können die kleineren Amplituden bei

hohen Frequenzen bei gleichen dB-Werten über der Hörschwelle in diesem Bereich unserer Ansicht aber nicht mit einer geringeren Lautstärke in Zusammenhang gebracht werden.

Als Ursache dieser Frequenzabhängigkeit der evozierten Potentiale ist die unterschiedliche Breite der Reizverteilung auf der Basalmembran (Kohlöffel, 1972) und damit unterschiedliche Anzahl der aktiven neuronalen Einheiten bei hohen und niedrigen Frequenzen wahrscheinlicher (Antono-Skinner, 1968).

#### Variabilität der Amplituden

In den Variationskoeffizienten der Abb. 7 sind sowohl der Versuchsfehler als auch tatsächliche Variabilität enthalten. Der Anstieg der Kurven bei kleinen dB-Werten resultiert daher zu einem Teil sicher aus einer größeren biologischen Streuung der Amplituden bei geringeren Reizstärken. Der andere Teil aber beeinflussen Mittelwertfehler die kleinen Amplituden bei geringeren Reizstärken relativ stärker. Die Auswertung wird schwieriger und ist mit einem größeren relativen Fehler behaftet.

Die von Karnahl & Stange (1972) gegebene Abhängigkeit des Variationskoeffizienten von der Reizintensität (ohne Spaltung in intra- und interindividuelle Variabilität) weist prinzipiell denselben Verlauf auf. Jedoch liegen die Werte dieser Autoren insgesamt niedriger. Die Ursache dieser Differenz ist eventuell die von Spreng & Karnahl (1967) angegebene Verminderung der Streuung durch Umrechnung der Amplitude in kortikale Erregung. Es mußte dann vor allem die interindividuelle Streuung vermindert werden, deren Anteil der Gesamtstreuung wesentlich größer ist.

Um eine statistisch gesicherte Aussage darüber machen zu können, ob die Potentiale verschiedener Personen einer Grundgesamtheit mit entsprechend großer Varianz zugeordnet werden können oder ob sie von

chen Populationen zuzuordnen sind, eine Varianzanalyse mit den 130 Werten der 13 Vp bei 50 dB durchgeführt. Varianzanalyse ergab mit einer Irrtumswahrscheinlichkeit von 1% Signifikanz, daß die Latenzen der einzelnen Personen unterschieden sich wesentlich.

An dieser Stelle muß betont werden, daß alle gemachten Aussagen nur auf die Studien beziehen, nicht auf den individuellen Potential Zeitverlauf. Eine solche Analyse wird zur Zeit durchgeführt.

Vergleich der Ergebnisse in Abb 4 mit von Stange (1973) veröffentlichten 500 Hz Latenzverläufen zeigt gute Übereinstimmung der N1 Latenzen. Das gilt für P2 Latenzen nicht. Während Stange im Bereich 20-80 dB eine Verkürzung der Latenz von etwa 65 ms feststellte, konnten wir nur mehr als 30 ms messen. Im Bereich 80-100 dB ändern sich die P2-Latenzen nach 40 dB nur noch geringfügig, bei Stange noch um mehr als 20 ms.

Wie von Benning et al (1972) gefundene Latenzwerte der 1000 Hz N1 Latenz zwischen 40-60 dB bestätigen unsere Messungen nicht. Die Verfasser fanden auch eine Altersabhängigkeit der Latenzen.

Vergleich nach Abb 4 die 500 Hz Latenzen mit den 1000 Hz Latenzen übereinstimmen und sich signifikant von den 4000 Hz Werten unterscheiden, fanden Benning (1972) keine Unterschiede zwischen den Latenzen bei 500 Hz und 4000 Hz.

Bei beiden zitierten Arbeiten anzunehmen, daß zufallsverteilte Reizung im Gegensatz zur regelmäßigen Reizung bei den vorliegenden Meßwerten kann die erwähnten Unterschiede nicht erklären, da nach unseren Ergebnissen (Sturzebecher et al 1974) bei einem mittleren Reizintervall keine wesentlichen Latenzdifferenzen bei regelmäßiger zufallsverteilter Reizung auftreten.

## Altersabhängigkeit

Aus Abb 7 ist ersichtlich, daß die Amplituden des AEP im untersuchten Altersbereich praktisch keine Altersabhängigkeit aufweisen. Dasselbe gilt auch für die hier nicht abgebildeten Latenzen.

## SUMMARY

The ERA characteristics (amplitudes and latencies) were measured in normal hearing adults (20 to 25 years of age) at frequencies of 500 Hz, 1000 Hz and 4000 Hz. Two other age groups (30 to 50 and 55 to 70 years of age) were measured only at 1000 Hz. Contrary to other authors, the linear scale was chosen to represent the



significant differences of amplitudes and latencies between the age groups could be found

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# MELANOCYTEN, LANGERHANSSCHE UND MERKELSCHES ZELLEN IN ORALEN EPITHELIIEN

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Die feinstrukturelle Morphologie von Melano-  
Langerhansschen und Merckelschen Zellen im nor-  
Epithel der dorsalen Zungenschleimhaut des  
ben wird elektronenmikroskopisch dargestellt  
cyten werden im normalen Papilleneithel regel-  
vorgefunden wenn klinisch auch keine Zeichen  
Pigmentierung vorliegen. Der basale epitheliale  
cyt enthält individuelle Pigmentorganellen ver-  
en der Melaninbiosynthese und ent-  
rudimentäre Halbdesmosomen gegen die Basal-  
na. Die morphologischen Besonderheiten der  
durch spezifische Organellen sowie das  
von Tonofilamenten und Desmosomen gekenn-  
an Langerhansschen Zellen deuten auf funk-  
Beziehungen zu Zellstrukturen der Keratinocyten  
epithelialen Keratinisationsprozeß. Die Mer-  
Zellen sind durch ihre horizontale Basallage  
in zu Keratinocyten. Fehlen von  
typische Merckelsche Granula und an-  
Nervenendigung charakterisiert. Die Beobach-  
von Merckelschen Granula mit dem peripheren  
der Merckel Zellen und die morphologische  
ast dieser Granula mit den Monoamin-  
ila bestimmter chromaffiner Zellsysteme  
Hinweise auf eine symbiotische Einheit von  
Merckelscher Zelle und assoziiertem Neu-  
mit neuroepithelialer Mechanorezeptorfunktion  
alschen Zellen erscheinen aufgrund ihrer spezi-  
Ultrastruktur als selbständige von Keratinocyten  
traditionellen Zellen unabhängige Zellpopulation  
Epithelien

Zu den intraepithelialen Nicht-Keratinocyten zählen Melanocyten, Langerhanssche und Merckelsche Zellen. Letztere Zellpopulation, von Merkel als „Tastzellen“ beschrieben (Merkel, 1875), ist erst in jüngster Zeit in das Blickfeld elektronenmikroskopischer Betrachtungen gerückt. Aufgrund ihrer Goldaffinität wurden die Langerhansschen Zellen (Langerhans, 1868) eine lange Zeit für neurogene Elemente gehalten. Nach Abtrennung von den Zellen des pigmentbildenden Systems, die erst in den letzten Jahren erfolgte, wird nunmehr eine mesenchymale Herkunft dieser Zellpopulation diskutiert. Funktion und biologische Dignität der Langerhansschen Zellen sind jedoch bis heute unbekannt.

Die Ultrastruktur der Keratinocyten des Papilleneithels der menschlichen Zunge ist an anderer Stelle (Schenk & Wersall, 1975) dargestellt worden. In der vorliegenden Arbeit werden elektronenmikroskopische Beobachtungen über das Vorkommen und die feinstrukturelle Morphologie von Melanocyten, Langerhansschen und Merckelschen Zellen im normalen Epithel der dorsalen Zungenschleimhaut des Menschen mitgeteilt.

## MATERIAL UND METHODEN

Biopsien normaler Schleimhaut der dorsalen Zungenoberfläche wurden von zehn Patienten, ohne Vorgeschichte oder klinische

mehrschichtige Plattenepithel keratinisiert. Mundhöhlenregionen stellt ähnlich der als eine symbiotische Einheit dar, die zu 90% aus Keratinocyten aufgebaut ist.

Unterstützung durch Fonds des Karolinska Institutet Stockholm

Zeichen einer Erkrankung der Mundhohlenschleimhaut, in Lokalanästhesie (Xylocain 0,5 %, Astra) entnommen

Die Gewebeproben zur Lichtmikroskopischen Untersuchung wurden in neutraler, gepufferte 7 % iger Formaldehyd Lösung fixiert, in Paraffin eingebettet und mit Hämatoxylin Eosin gefärbt

Die Präparate zur elektronenmikroskopischen Untersuchung wurden in kalter 1 % iger Osmiumtetroxyd Lösung, gepuffert mit Veronalacetat auf pH 7,4, fixiert, dehydriert und in Epon eingebettet. 1–3 µm dicke Schnitte wurden mit Toluidinblau gefärbt und mit dem Lichtmikroskop orientiert. Ultradünnschnitte (300–500 Å) wurden mit einem LKB Ultramicrotom angefertigt und auf Kupferobjektträger montiert. Die Nachkontrastierung erfolgte mit Uranylacetat (Watson, 1958) und mit Bleicitrat (Reynolds, 1963). Die elektronenmikroskopische Untersuchung der Präparate wurde mit einem Siemens Elmiskop I bei einer Strahlenspannung von 80 kV durchgeführt.

### Melanocyten

Melanocyten kommen als regelmäßiger Bestandteil des Zungenepithels zur Beobachtung, wenn klinisch auch keine Anzeichen einer Pigmentierung gegeben sind (Abb. 1a). Der Kern dieser dendritischen Zellen ist meist weniger tief eingebuchtet, als dies bei den Langerhansschen Zellen der Fall ist, und kann bisweilen eine mehr abgerundete Form annehmen. Die charakteristische Organelle dieser Zellpopulation und zugleich Endprodukt der Melaninbiosynthese ist das ca.  $0,3 \mu\text{m} \times 0,1 \mu\text{m}$  große, je nach Schnittebene runde, ovale oder langliche Melanosom mit homogenem elektronendichten Inhalt und äußerer, nicht immer erkennbarer Membranbegrenzung. Die Prämelanosomen, die als Vorstadien der Melanosomentwicklung gelten, besitzen eine granulare, spiralförmige oder striäre Innenstruktur mit einer 90–100 Å Periodizität und einer dem Reifegrad der Melanogenese entsprechenden Elektronen-

dichte (Abb. 1b). Während der Auszumelanosom kommt es durch zunehmende Pigmentablagerung zu einer Maskierung der periodischen Innenstruktur. Der Granulierung der Melanosomen werden von dem Cytoplasma der Melanocyten bzw. ihrer Zellenditen in das Cytoplasma der benachbarten Keratinocyten transferiert, als individuelle Pigmentorganellen in Form von mindestens 2–3 Melanosomenkomplexen vorgefunden.

Das kontrastarme Cytoplasma der Melanocyten enthält neben zahlreichen Ribosomen und Mitochondrien spärlich elektronendichte plasmatische Filamente geringer Elektronendichte und ohne Neigung zur Bündelung. Golgi Apparat und endoplasmatisches Reticulum sind gut auspräpariert. Der in den basalen und suprabasalen Schichten vorkommende Melanocyt ist gegen das Cytoplasma der angrenzenden Keratinocyten häufig durch cytoplasmatische Fortsätze abgegrenzt. Desmosomale Verbindungen zwischen Melanocyten oder anderen dendritischen Zellen kommen nicht vor, gegen das Cytoplasma der Keratinocyten können jedoch modifiziert desmosomartige Strukturen an der peripheren Zellgrenze entwickelt werden.

### Langerhanssche Zellen

Langerhanssche Zellen kommen im Epithel der menschlichen Zunge regelmäßig im Stratum spinosum vor und besitzen die ultrastrukturellen Merkmale der entsprechenden dendritischen Zellen der Epidermis (Abb. 2a). Das kontrastarme Cytoplasma dieser dendritischen Zellen ist meist kontrastärmer als das der angrenzenden Keratinocyten und enthält neben elektronendichten plasmatischen Filamenten auch Mitochondrien. Neben den des Melanocyten keine 1 µm zur Aggregation aufweisen. Neben Mitochondrien, Ribosomen, einem gut entwickelten Golgi Apparat und endoplasmatischem Reticulum finden sich Lysosomen und Vakuolen, Lipideinschlüsse und



Abb 1a Melanocyt mit zahlreichen Pigmentorganellen in der Basalzellschicht des dorsalen Zungenepithels V Zellkern S Sphaerion G Golgi Zonen, ICR Interzellulär BM geschichtete Basalmembran KC Keratinozyten mit cytoplasmatischen Ausläufern

Abb 1b Premelanosomen in verschiedenen Schnittebenen mit granularer spiraler oder striärer Innenstruktur und deutlich erkennbarer dreischichtiger Membran Die Elektronendichte der Pigmente variiert entsprechend dem Reifestadium



**Zeichen von mitotischer Aktivität** Die für diese Zellenart typischen Organellen sind die bis zu 1  $\mu\text{m}$  langen, stabförmigen Langerhansschen Granula, die von parallelen Membranen begrenzt werden und eine zentrale doppelreihige Lamelle sowie je eine randständige einreihige Lamelle elektronendichter 50 Å-Partikel mit einer 90 Å-Periodizität enthalten (Abb 2c). Bei dem Tennisschlagertyp der Langerhans-Granula besitzt das Endstück einer Seite eine vesikuläre Erweiterung. Bei flachem Anschnitt der in der dritten Dimension schalen- oder scheibensförmigen Gebilde wird eine parakristalline Innenstruktur sichtbar (Abb 2c). Langerhanssche Granula wurden im Papilleneithel vereinzelt in Kontakt und auch in Kontinuität mit der Plasmamembran gefunden (Abb 2b). Der längliche, eher grazil gebaute Zellkern besitzt die für diese Zellenart charakteristischen tiefen Einbuchtungen seiner Kernmembran.

**Desmosomale Zwischenzellkontakte** zu Keratinocyten oder anderen intraepithelialen Zellen werden nicht entwickelt. Cytoplasmatische Vorstülpungen der Langerhansschen Zellen in entsprechende Invaginationen der angrenzenden Keratinocytenmembran sind jedoch häufig nachweisbar und konnten für einen intensiven funktionellen Kontakt zwischen den beiden Zellpopulationen sprechen. Langerhanssche Zellen fanden sich häufig in unmittelbarer Nähe von interzellulären Ansammlungen mikrovillöser Cytoplasmafortsätze, die mit zahlreichen Desmosomen behaftet waren (Abb 2a). Bei Kontaktaufnahme mit Langerhansschen Zellen kam es in den peripheren Cytoplasmaabschnitten benachbarter Keratinocyten häufig zu einer Retraktion und Invagination von Desmosomen sowie zu einer massiven Konzentration und räumlichen Ausrichtung von cytoplasmatischen Tonofilamenten. An anderen Stellen wurden hingegen umschriebene tonofilamentfreie, organellen und kontrastarme, periphere Cytoplasmazonen in angrenzenden Keratinocyten beobachtet. Die Anzahl der Langerhans-Zellen war in starker

keratinisierten Regionen deutlich größer, dem an das Papilleneithel grenzende Gangseithel mit geringer Verteilungstendenz.

### Merkelsche Zellen

Die Merksche Zelle liegt mit ihrer Achse meist parallel zur elektronenmikroskopischen Basalmembran und ist gehäufig durch Cytoplasmafortsätze benachbarter Keratinocyten abgegrenzt. Allen Merkschen Zellen ist intraepithelial eine diskusartige myelinfreie Nervenfaser mit zahlreichen Mitochondrien und angelagert. Regelmäßiger findet man Nervenendigungen im subepithelialen Gewebe in unmittelbarer Nähe der Merkschen Zelle. Der längliche Zellkern oft tiefe Invaginationen der Kernmembran. Das Cytoplasma der Zellen, das in seinem Kontrastarmut den beschriebenen dendritischen Zellen vergleichbar ist, verlaufen feine plasmatische Filamente, die keine Tendenz zur Aggregation aufweisen. Das ultrastrukturelle Erkennungszeichen sind die Merkschen Granula, 600–1500 Å groß, membranbegrenzte, rundliche bis ovale Gebilde. Das elektronendichte Innere dieser Granula ist von der Außenmembran durch eine kontrastarme Zone getrennt (Abb 3b). Größe, Zahl und Elektronendichte der Granula variieren vermutlich entsprechend dem Reifezustand. Granulaformen mit einem Durchmesser an der unteren Grenze der genannten Größenordnung besitzen eine kontrastärmere granular filamentöse Innenstruktur und/oder einen exzentrischen Kernanteil. Gelegentlich sind bei Kontaktaufnahme oder Fusion der Granula mit dem peripheren Plasmalemma Beobachtungen (Abb 4a, b). Bei polarisierter Lichtmikroskopie ist der Golgiapparat an diesen Regionen abgewandete des Zellkernes gelegen.

Die voluminösen Mitochondrien.



Abb 2a Langerhanssche Zelle (LH) mit tieferen Zellkern (N) und dendritischem Cytoplasma (D) im Stratum spinosum des Papillarschichtes mit mikrovillösen Epithelfortsätzen in den Zellzwischenraum angrenzende Keratinocyten

Abb 2b Dendrit einer Langerhans Zelle = Q

schnell Langerhanssche Granula (Pfeile) in Kontakt mit der Plasmamembran

Abb 2c Vergrößerte Ausschnitt aus Abb 2a Stabförmige Langerhanssche Granula (LG) mit periodischer Lamellenstruktur Parakristalle (Pfeile) bei hohem Anschnitt der Zellkern

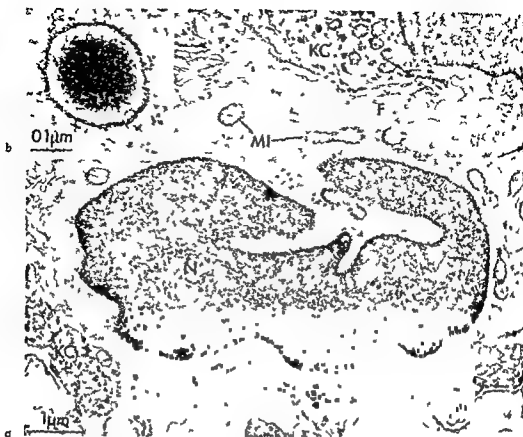


Abb 3a Merckelsche Zelle in typischer horizontaler Basallage. Eingebuchteter Zellkern (N), voluminöse Mitochondrien (MI), Merckelsche Granula (Pfeile) und cytoplasmatische Filamente (F). Keratinocyten (KC) mit Cytoplasmafortsätzen. Basalmembran (BM) an der Bindegewebsgrenze.

Abb 3b Merckelsches Granulum bei größter Vergrößerung. Der elektronendichte innere Kern von granular filamentöser Beschaffenheit erstreckt sich in die d = Kontrast und erreicht an manchen Stellen die e = Referenz der dreischichtigen Außenmembran.

mäßig geringer als in Keratinocyten, zahlreiche Ribosomen-Partikel und granulares endoplasmatisches Reticulum sind im Cytoplasma verteilt. Daneben findet man oft zahlreiche cytoplasmatische Vesikel, multivesikuläre Körper, Lysosomen und vereinzelt Centriolen. In der Cytoplasmaperipherie sind stellenweise Zeichen einer hohen pinocytotischen Aktivität vorhanden. An der lateralen und distalen Plasmamembran sind vereinzelt schwach ausgebildete desmosomartige Kontakte zu Keratinocyten nachweisbar. Die mit der Haftplatte der desmosomalen Strukturen assoziierten Filamente sind ausserst spärlich ausgebildet oder fehlen zur Ganze. Zellverbindungen zu dendritischen Zellen oder anderen Zellen als Keratinocyten

sowie zur Basalmembran werden nicht beobachtet.

#### Übrige intraepitheliale Zellen

Neben den erwähnten Zellpopulationen im Papillenepithel der Zunge finden sich dendritischen Zellen ähnliche Zellen in basaler und suprabasaler Lage, die in ihrem Cytoplasma beschriebenen spezifischen Organellen enthalten. Es könnte sich dabei um geschulten Zelltypen handeln, die charakteristische Organellen außerhalb der betrachteten Schnittebene oder in dendritischen Zellfortsätzen lokalisiert sind, wo sie durch Seneschritte nicht immer aufzufinden sind. Andererseits könnte sich die Beur-



Fusion eines Merkschen Granulums mit dem am der Merkel Zelle Vergrößerter Ausschnitt

Merkelsche Zelle mit Merkel-Granula (MG)  
Abbildung obere Granulum in Fusion mit der

Plasmamembran Eingebuchteter Zellkern (N) voluminöse Mitochondrien (MI) ICR Interzellularraum Durch Seneschritte konnten in dieser Zelle weitere Merkel-Granula und Desmosomen aufgedeckt werden

sche Zelle in einem Entwicklungs-  
unktionszustand befinden, bei dem  
fischen Organellen tatsächlich nicht  
en oder mit den verwendeten präpara-  
ethoden nicht nachweisbar sind Falls  
ber nicht beschriebene dritte Art von  
schen Nicht Keratinocyten existieren  
so liegen bislang keine ausreichenden  
ktuellen Kriterien vor, die eine  
rierung einer solchen Zellpopulation  
n wurden

Vorkommen von intraepithelialen Ner-  
bungen wurde bereits oben erwähnt  
halb des Niveaus der Merkschen Zel-  
den sich freie Nervenendigungen im  
ellularraum oder in Plasmamembran-  
tionen von Keratinocyten Das hoch-  
isierte Epithel der Geschmacks-  
uagenden Zungenpapillen kann hier  
hahnt werden Die Einwanderung von

verschiedenen Zellen wie Lymphocyten,  
Granulocyten, Makrophagen usw aus dem  
subepithelialen Bindegewebe ist auf Einzel-  
beobachtungen beschränkt und kann eher als  
die Ausnahme zur Regel gelten Das intra-  
epithelial Auftreten von Mastzellen, das  
unter pathologischen Bedingungen vorkom-  
men kann, ist ebenfalls kein regelmäßig zu  
beobachtendes Charakteristikum des nor-  
malen Papilleneithels der menschlichen  
Zunge

## DISKUSSION

Über Melanocyten, Langerhanssche Zellen  
und Merkel Zellen des Papilleneithels der  
menschlichen Zunge liegen bisher keine elek-  
tronenmikroskopischen Untersuchungen vor  
Hutchens et al (1971) untersuchten die Ver-  
teilung der dendritischen Zellen in oralen

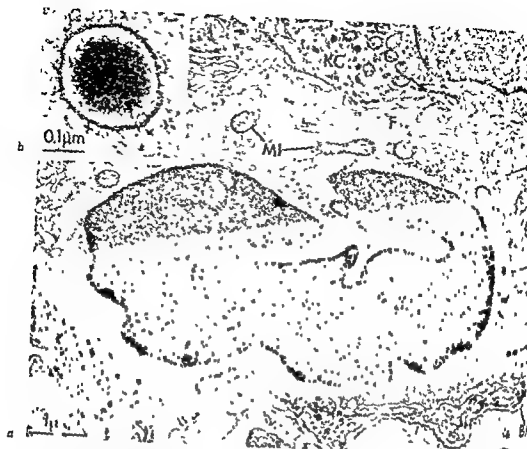


Abb 3a Merksche Zelle in typischer horizontaler Basallage Eingebuchteter Zellkern (N), voluminöse Mitochondrien (Mf), Merksche Granula (Pfeile) und cytoplasmatische Filamente (F), Keratinocyten (KC) mit Cytoplasmafortsätzen, Basalmembran (Bf) an der Bindegewebsgrenze

Abb 3b Merksches Granulum bei 2. Vergrößerung Der elektronendichte innere Kern von granular filamentöser Beschaffenheit. Elemente erstrecken sich in die kontrastärtere und erreichen an manchen Stellen die inneren Schichten der dreischichtigen Außenmembran

mäßig geringer als in Keratinocyten, zahlreiche Ribosomen-Partikel und granulares endoplasmatisches Reticulum sind im Cytoplasma verteilt. Daneben findet man oft zahlreiche cytoplasmatische Vesikel, multivesikuläre Körper, Lysosomen und vereinzelt Centriolen. In der Cytoplasmaperipherie sind stellenweise Zeichen einer hohen pinocytotischen Aktivität vorhanden. An der lateralen und distalen Plasmamembran sind vereinzelt schwach ausgebildete desmosomartige Kontakte zu Keratinocyten nachweisbar. Die mit der Haftplatte der desmosomalen Strukturen assoziierten Filamente sind äußerst spärlich ausgebildet oder fehlen. Zur ganzen Zellverbindungen zu dendritischen Zellen oder anderen Zellen als Keratinocyten

sowie zur Basalmembran werden nicht wickelt.

#### Ubrige intraepitheliale Zellen

Neben den erwähnten Zellpopulationen im Papillenepithel der Zunge sind dendritischen Zellen ähnliche, konzentrische Zellen in basaler und suprabasaler Lage getroffen, die in ihrem Cytoplasma charakteristische Organellen enthalten. Es könnte sich dabei um die geschilderten Zelltypen handeln, deren charakteristische Organellen außerhalb der betrachteten Schnittebene oder in dendritischen Zellfortsätzen lokalisiert sind, wo sie durch Serienschritte nicht immer aufzufinden sind. Andererseits könnte sich die be-

und bei Vitiligo (Ebner & Niebauer, 1967) und von uns in Plattenepithelcarcinomen (öffentliche Befunde) regelmäßig gesehen. Ein dreidimensionales Modell der Langerhans-Granula wurde von Wolff (1967b) beschrieben. Der Kontakt oder Kontinuität der Granula mit der Plasmamembran wurde in unseren Präparaten im unserem Material gesehen (Abb. 2b), viel häufiger war jedoch eine Kontinuität mit Golgi Elementen gegeben, was auf die Entstehung aus dem Golgi Apparat hindeutet (Zelickson, 1965, Wolff, 1967b, & Schreiner, 1970). Von anderen Autoren wurde, zum Teil ebenfalls auf Tracer- und Enzymmarkierungen gestützt, eine entgegengesetzte, eine zentripetale Wanderung und ein endocytotischer Entstehungsmechanismus der Langerhans Granula. Abschnürung eingestulpter Plasmamembranteile postuliert (Tarnowski & Hashimoto, 1967, Hashimoto & Tarnowski, Hashimoto, 1970).

Die spezifische Ultrastruktur der Langerhansschen Zelle deutet auf eine aktive, zur Differenzierung fähige und mit Spezialfunktionen ausgestattete Zelle. Ein funktioneller Zusammenhang mit dem Keratinisationsprozeß wird in unserem Material durch Vermehrung der Langerhans Zellen in Epithelien mit höherem Differenzierungsgrad deutlich. In unverhornten Epithelien der Ratte wurden Langerhanssche Zellen nach Vitamin A Entzug gleichzeitig mit dem Auftreten von keratinproduzierenden Metaplasien gefunden (Wong & Buck, 1967). Beim Rhesus-Affen war in den keratinisierten Epithelien der Mundhöhle die Anzahl der Langerhans Zellen höher als in nicht-keratinisierten Epithelien und am größten in dem obersten Epithel des Zungenrucks (Schreiner et al., 1971). Eine Phagocytosefunktion scheint nicht die Haupteigenschaft der Langerhansschen Zellen zu sein. Wir haben in pathologischen Material (unveröffentlichte Befunde) phagocytisierte Zellreste von Langerhans Zellen im Cytoplasma benachbarter Keratinocyten gesehen. Eine definitive Funktion dieser Zellen erscheint

uns über eine Wirkung auf Desmosomen, Tonofilamente und Zellmembranen von Keratinocyten eine Beeinflussung des Differenzierungs- und Keratinisationsprozesses verhornter Epithelien zu sein.

Die Merckelschen „Tastzellen“, denen mechanorezeptorische Funktionen zugeschrieben werden, kommen in der Basalschicht des Papillenepithels vereinzelt oder in Gruppen von mehreren Zellen vor. Kontrastarmes Cytoplasma, Merckel-Granula, Desmosomenkontakte und angelagerte Nervenendigung sind charakteristische Merkmale dieser Zellen. Kurze, zapfenartige Cytoplasmavorstülpungen erstrecken sich zwar gegen angrenzende Keratinocyten und breitbasige Cytoplasmavorstülpungen gegen andere, benachbarte Merckel Zellen, die Ausbildung von langen, dendritischen Zellfortsätzen ist jedoch nicht zu beobachten, weshalb die Merckelschen Zellen nicht als dendritische Zellen bezeichnet werden können.

Über Merckel-Zellen im Epithel der Gingiva wurde von Hashimoto (1972) berichtet, „gut entwickelte Bündel von Tonofibrillen“ konnten wir in den Merckel-Zellen unseres Materials nicht beobachten. Von Fortman & Winkelmann (1973) dargestellte filamentöse Kerneinschlüsse sind nach unseren Befunden nicht kennzeichnend für diese Zellenart, sie konnten jedoch Hinweise auf eine neurogene Herkunft der Merckelschen Zellen geben. Ähnliche Gebilde wurden in Zellen des Cerebralganglions von Enchytraeus nach Puromycingabe gesehen (Gersch & Ude, 1971). Das Vorkommen einer angelagerten Nervenendigung im Niveau der Merckelschen Zelle wurde bei verschiedenen Säugetieren und beim Menschen beschrieben (Munger, 1965, Mustakallio & Kustala, 1967, Smith, 1970, Suzuki & Kurosaki, 1972), ist aber in der oralen Mucosa (Fortman & Winkelmann, 1973) zumindest kein regelmäßig zu beobachtendes Charakteristikum dieser Zellpopulation.

Eine Fusion der Merckelschen Granula mit dem Plasmalemma (Abb. 4a, b) konnte als morphologisches Anzeichen einer Entleerung

und Abgabe des Granulainhaltes in den extrazellulären Raum gedeutet werden. Die einzigen Hinweise auf eine Fusion der Merkel-Granula mit der Plasmamembran ergaben sich bisher in den dermalen Grandryischen Körperchen von Embryonen der Pekingtonente (Saxod, 1970) und in der Buccalmucosa der Ratte (Chen et al., 1973). Den Merckelschen Granula ähnliche „dense-cored vesicles“ konnten kürzlich in neuroepithelialen Körperchen der Bronchial- und Bronchiolarmucosa von Kaninchen gefunden und ihr Gehalt an Serotonin nachgewiesen werden (Lauweryns et al., 1973).

Die morphologische Verwandtschaft der Merckelschen Granula mit den Catecholamingranula der Zellen des Nebennierenmarkes, sympathischer Ganglien und anderer chromaffiner Gewebe, sowie der Nachweis von synaptischen Strukturen in Merckelschen Endigungen verschiedener Mammalia (Andres, 1966, Halata, 1973, Chen et al., 1973) deuten auf eine funktionelle Einheit von spezialisierter Merkelzelle und assoziierter Nervenendigung. Mechanische Impulse, die auf das Epithel einwirken, würden dabei durch Cytoplasmafortsätze und Desmosomen von der Merckelschen Zelle aufgenommen, transformiert und durch extrazelluläre Abgabe einer Transmittersubstanz aus den Merkel-Granula auf die sensorische Nervenendigung übertragen.

Die Bestätigung dieser Hypothese sowie die Aufklärung der chemischen Beschaffenheit der Merckelschen Granula muß physiologischen, pharmakologischen, mikrospektrographischen und elektronenmikroskopisch-cytochemischen Untersuchungen vorbehalten bleiben. Die spezifische Ultrastruktur dieser Zellenart veranlaßt uns zu der Auffassung, daß die Merckelschen Zellen eine selbständige, von Keratinocyten und dendritischen Zellen unabhängige Zellpopulation oraler Epithelien darstellen.

Für ihre wertvolle technische Assistenz sei Fräulein Ann Mane Lundberg und Herrn Bengt Hedberg herzlich gedankt.

## SUMMARY

The fine structural morphology of melanocytes and Merkel cells in the normal dorsal skin of the human tongue has been studied by electron microscopy. Melanocytes were regularly found in the

dermal papillae and rudimentary half-desmosomes between the melanocyte and the basal most suprabasal Langerhans cells can be identified. Specific organelles and by the lack of tonofilament desmosomes. The morphological features of keratinocytes suggest functional relations to cell structures of keratinocytes and to the epithelial keratinization process. The Merkel cell is characterized by a horizontal orientation in the basal epithelial layer, the development of desmosomal attachments to keratinocytes, the lack of tonofilaments, the typical Merkel cell granules and an

epithelial tactile receptor function. Because of its specific ultrastructure, the Merkel cells are thought to constitute a self-maintaining cell population independent of keratinocytes and dendritic cells in the oral

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## SURVIVAL AFTER HAEMORRHAGE FROM THE BRACHIOCEPHALIC TRUNCUS FOLLOWING TRACHEOSTOMY

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**Abstract** A patient with two massive haemorrhages from the brachiocephalic truncus after tracheostomy is reported. An analysis of 6 previously reported long term survivors following this complication shows that the initial care in controlling the haemorrhage is of major importance. The operation method of choice is permanent division of the brachiocephalic truncus. Transitory or no neurological postoperative complications were found.

The most dangerous and dramatic complication after tracheostomy is the delayed, massive haemorrhage from the brachiocephalic truncus. Of all the complications following tracheostomy, this unforeseen catastrophe has the highest mortality rate (Mathog et al., 1971). Considering the increased number of tracheostomies performed, resulting from the widening indications for tracheostomy, the frequency of this complication is on the increase (Bihler & Hutschenreuter, 1966, Comer et al., 1974). Not until 1968, was the first long-term survivor reported by Reich & Rosenkrantz (1968). To the best of our knowledge, only 6 patients have previously been recorded as surviving after a haemorrhage from the brachiocephalic truncus and after leaving hospital.

We thought it worthwhile to report on a patient who survived two such haemorrhages but later expired. In order to contribute to a better prognosis for patients suffering from this kind of haemorrhage, an analysis was

made both of the case and the, reported long term survivors. Circumstances, which may have contri- survival, are discussed.

### *Case report*

The patient was a 60-year-old woman, afflicted by advanced rheumatoid arthritis with a pronounced kyphoscoliosis, admitted to the hospital suffering from expiratory stridor and increasing respiratory insufficiency due to complete paralysis of the left vocal cord and partial paralysis of the right vocal cord. Tracheostomy was performed. She received a Portex tracheostomy tube which was exchanged for a silver cannula one week. The silver cannula had to be replaced by a Rüsch tube, because of a narrowing of the trachea, which caused a partial intermittent obstruction of the trachea. Several times the tube was dislodged from the tracheostoma due to violent coughing.

Nine months after the tracheostomy was performed, a slight haemorrhage occurred from the tracheostoma. However, it stopped spontaneously. A sudden, unexpected massive haemorrhage occurred 4 days later. The tracheostomy tube was routinely changed. The tube was immediately reintubated, the cuff was inflated and the haemorrhage was controlled. An erosion of the brachiocephalic truncus was now suspected. The patient was taken to the operating

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*Fig 1* Frontal view of the superior thoracic outlet. The aortic arch (A) is displaced to the left. The brachiocephalic truncus (T) leaves the aortic arch dislodged to the left of the midline and transverses abnormally high in the thoracic cavity. The close relationship between the inferior concave side of the tracheostomy tube and the brachiocephalic truncus is seen.

tracheostoma was carefully inspected only a new massive haemorrhage appeared. This could temporarily be controlled by digital compression through the lower part of the tracheostoma against the sternum. Another tracheal intubation was performed during general anaesthesia and a median sternotomy was performed. This created a good access to the suspected area of haemorrhage. A defect in the vessel wall was found in the wall of the brachiocephalic truncus, near the lower border of the tracheostoma, where the truncus and the trachea were in close spatial relationship to each other. The defect in the vessel wall was sutured. The tracheostoma was closed upwards and the cricoid cartilage was used to create a better lodge for the tracheostomy tube. A Portex tracheostomy tube was inserted. The postoperative course was unremarkable. Thirteen days later another sudden massive haemorrhage from the tracheostoma occurred which could be temporarily controlled by im-

mediate inflation of the cuff. At operation a new erosion of the vessel in the formerly sutured area was found. After resection of a small necrotic part of the vessel wall, the defect was closed by sutures. No division of the artery was done and no graft was inserted. Postoperatively the patient was treated by artificial respiration through an endotracheal tube. She died 10 days later due to respiratory and circulatory insufficiency.

#### *Autopsy*

**Macroscopical findings** A pronounced kyphoscoliosis in the thoracocervical region with several collapsed vertebral bodies were found. Moderate to severe arteriosclerosis was noticed in the coronary vessels. The myocardium showed slight fibrosis. In the pericardial sac, fibrin adhesions were found. The lungs, liver, spleen and kidneys showed massive blood congestion.

The lower border of the tracheostoma was in close relationship to the brachiocephalic



Fig 2 Low power photomicrograph showing a portion of the mucosa (M) and cartilage (C) of the trachea and the elastic membranes (E) in the wall of the brachiocephalic truncus. The elastic fibres are split up and the arterial wall is replaced by granulation tissue (G). Thrombotic material is adhering to the luminal surface of the granulation tissue. van Gieson-elastic  $\times 10$ .

truncus which left the aortic arch to the left of the midline and crossed the trachea at an abnormally cranial level (Fig 1). At the cranial surface of the vessel approximately 3 cm from the aortic arch, disintegrating blood clots and granulations covered the sutured area. No communication was found to the lumen of the artery. On the corresponding intimal surface of the vessel wall thrombotic material was found.

**Microscopical findings.** Granulation tissue and loose connective tissue with scattered inflammatory cells were found between the brachiocephalic truncus and the tracheal wall in the sutured area. In the vessel wall degeneration of the elastic fibres was seen. In some parts the vessel wall was totally necrotic and replaced by granulation tissue. At the luminal surface of the granulation tissue a thrombus was present (Fig 2).

## DISCUSSION

Almost every case of massive haemorrhage as a complication of tracheostomy presents as a complete surprise to the clinician. A fulminating haemorrhage may occur at any time but seldom more than one month after the tracheostomy. Hutschenreuter (1966) Brantigan (1966) and one case has been reported occurring postoperatively (Weissman 1974). The cephalic truncus is the most commonly involved vessel (Bihler & Hutschenreuter 1970). No patient is said to be predisposed to this complication. It is of the greatest importance how to handle this type of haemorrhage. In 50% of the patients exsanguinate airways in seconds if an immediate ligation of the haemorrhage is not obtained (Ebert 1970).

In our case the haemorrhage occurred late as 9 months after the tracheostomy. Obviously the thoracic deformity caused by the placement of the aortic arch resulted in an abnormal cranial position of the cephalic truncus. Anomalous position of the major vessels has previously been reported as a cause in the pathogenesis of the complication (Scheldrup 1957, Weir 1964, 1973). Jarvis (1964) reported a posterior origin of the brachiocephalic truncus of the midline similar to that reported in our patient. Occasionally the truncus is found at the level of the second and third thoracic vertebrae (Willerson 1965) and the close proximity of the inferior concave surface of the ostomy tube thus predisposes to the complication caused by constant pounding of the tube against the tracheostomy tube (Baker 1970, Utley et al 1977). A similar complication has obviously been reported in the literature (Silén 1970). A necrotic

# 1 Review of 6 previously reported long-term survivors after massive erosion haemorrhage in brachiocephalic truncus following tracheostomy

erosion means a mechanism of vascular erosion caused by the tip of the tracheostomy tube with necrotizing action and secondary penetration through the tracheal wall. Direct erosion means a mechanism of vascular erosion caused by a constant pounding of the vessel wall against the inferior concave surface of the tracheostomy tube.

Patient	Symptoms prior to haemorrhage	Post-operative interval (days)	Pathogenesis	Initial care	Operative method	Comment
14 year old girl	None	225	Indirect erosion	Cuff inflation	Sternotomy division	No neurological complications
70-year old woman	Hemoptysis	18	Direct erosion	Tube reinsertion digital compression endotracheal intubation	Sternotomy division	Haemorrhage at decannulation no neurological complications
21 year old girl	None	9	Direct erosion	Tube reinsertion	Sternotomy division	Haemorrhage at decannulation slight weakness of the arm
11 year old girl	None	7	Direct erosion	Endotracheal intubation digital compression	Sternotomy division	No neurological complications
11 year old girl	None	6	Direct erosion	Digital compression tube reinsertion	Suprasternal approach division	No neurological complications
27 year old man	None	340	Direct erosion	Tube reinsertion digital compression	Sternotomy division	Haemorrhage at decannulation no neurological complications

to an irritating trauma by the tip of the tube. This type of vascular erosion is regarded as the most important mechanism in the formation of a tracheo-arterial fistula (Lunding, Reich & Rosenkrantz, 1968, Mathog et al, 1971). Only one patient has been reported surviving this type of arterial erosion (Table 1, Reich & Rosenkrantz, 1968).

The classical warning symptoms prior to massive haemorrhage are of importance. A self-limiting haemorrhage a few days before the major haemorrhage has been reported (Rogers, 1969, Biller & Ebert, 1970, Ebert, 1973). This was also noticed in the patient reported here. A pulsating tube (Willerson, 1965), and a feeling of suprasternal discomfort together with an irritating cough can be present before a massive haemorrhage (Jormolund, 1962).

The frequency of serious, delayed haemorrhages induced by erosion of the brachiocephalic trunk

(Rogers, 1969, Kantawala et al, 1972). At the University Hospital of Umeå, Sweden, 1000 tracheostomies have been performed during the last 10 years and 2 cases of tracheostomy tube erosion haemorrhage have been registered. However, this figure probably is too small since a fistula between the trachea and the artery can be rather difficult to find, not only at operation, but also at autopsy (Willerson, 1965, Weissman, 1974).

The total incidence of all types of haemorrhage after tracheostomy is about 1-3% (Rogers, 1969, Mathog et al, 1972, Kantawala et al, 1972). The early postoperative slight oozing of blood from the tracheostoma is due to inadequate hemostasis (Willerson, 1965, Rogers, 1969). Only a few hours after a tracheostomy, a developing dry haemorrhagic tracheobronchitis can be observed (Pfretzschner et al, 1973). Concerning the sudden massive haemorrhage from a different

diagnostical considerations should be avoided. All postoperative haemorrhages should be looked upon as if they were prodromal signs of early erosion of a major neck vessel.

A review of the 6 cases of long-term survival after haemorrhages from the brachiocephalic truncus (Table I) shows that the haemorrhages started at the time of decannulation in 3 cases. By analysing the initial care of these 6 survivors, it can be recommended to recannulate the patient immediately, if the haemorrhage occurs by the time of decannulation. Inflation of the cuff should also be done. According to Utley et al (1972), the initial inflation of the cuff is unsuccessful in controlling the haemorrhage in most patients, but likewise the initial procedure should be a cuff inflation in order to prevent aspiration. If it is not possible to cuff the tube or control the haemorrhage by inflation of the cuff, insertion of the index finger through the tracheostoma is recommended, in order to compress the brachiocephalic truncus against the sternum. After this an endotracheal intubation can be performed (Table I). Restoration of blood volume prior to operation is of great importance to reduce postoperative neurological complications, which are expected to appear in 20–30%, when using the recommended operation method with division of the brachiocephalic truncus (Biller & Ebert 1970). However, a minor postoperative neurological complication was reported in only one of the long term survivors. Thus the apparent operative method of choice after a median sternotomy is permanent division of the truncus (Table I). This method was used in all long term survivors.

### ZUSAMMENFASSUNG

Ein Patient mit zwei massiven Blutungen aus dem Truncus brachiocephalicus nach einer Tracheostomie ist beschrieben worden. Eine eingehende Analyse von sechs früher beobachteten Überlebenden zeigt, dass eine frühzeitige und adäquate Kontrolle der Blutung von grosser Bedeutung ist. Die gewählte Operationsmethode ist eine endgültige Teilung des Truncus brachiocephalicus. Vorübergehende oder neurologisch postoperative Komplikationen waren nicht vorhanden.

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## THE PROBLEM OF UNILATERAL AND BILATERAL NECK DISSECTION

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270 unilateral and bilateral neck resections have been performed in different primary localisations of the head and the neck at the Ear Nose and Throat Department of the Medical Faculty in Zagreb in the period 1960-70. Of 209 patients with unilateral resection 178 survived (37%) while only 3 patients (4.9%) died of a total of 61 patients where bilateral resection was performed. An analysis is given separately of radical neck dissection in carcinoma of the larynx, the hypopharynx and the thyroid. From this analysis and from immunologic considerations the following conclusions are derived: (i) The results are not in favour of prophylactic block resection of the lymph nodes, which would be contrary to our knowledge of the role of the lymph nodes in the struggle against the malignant lesion. (ii) Evacuation of the neck lymph nodes is performed in carcinoma of the larynx, the hypopharynx and the thyroid carcinoma as histologically positive lymph nodes are usually found in this site in large numbers.

The problem of radical neck dissection became a focus of attention in 1906 when Crile published his work "Excision of the Head and Neck in America". A large number of cases were reviewed in this work, i.e. 4500 patients dying from carcinoma of the head and neck, 10% had died because of an inadequately performed primary tumour or metastases in the neck, while only 1% of deaths were due to distant metastases. The hope aroused by radiotherapy slightly checked the enthusiasm for surgery but it very soon became apparent that metastases in the neck were resistant to radiation and in a great number of cases the percentage of recoveries was accordingly low, therefore the advocates of surgical therapy

prevailed once more, whereby the American authors MacComb (1969), Martin & Morfit (1944), and Conley (1967) contributed most and radical neck resection became the subject of interest not only for otorhinolaryngologists but for a large group of head and neck surgeons too.

Moreover it became not only the method of choice but a sort of prophylaxis against the spreading of carcinoma, because some authors as, e.g. Willis (1952) maintained that the lymph nodes not only are no obstacle to but even represent fertile soil for the development of carcinoma. Thus in the course of time an ever increasing number of authors in the ear, nose and throat branch of medicine began to favour the idea that, e.g., in laryngeal carcinoma as the most frequent malignant localization it is necessary to carry out parallel block resection of the neck in all cases except in localizations on the vocal cords.

Twenty-two out of a number of 38 otorhinolaryngologists from different countries were in favour of this solution in an enquiry conducted by the Milan School of Medicine prior to the International Congress of Paris in 1961. The first report on radical neck resection in Yugoslavia was given by our Department in 1956 at the Congress for Maxillofacial and Plastic surgery, when it was pointed out that we could not possibly favour prophylactic block resection since by removing the lymph

tissue of the neck it was not possible to change the relationship between the malignant lesion and the immunologic forces of the organism. Subsequently our standpoint was strengthened as, by studying the relationship between the immunoglobulins and the malignant tumour, we discovered that each malignant lesion inevitably engaged the entire defensive system and that on this relation alone the problem of prophylactic block resection should be viewed.

Over the last few years immunology rapidly became involved with the problems of cancer and we have now valuable data at our disposal concerning this sphere of activity.

### CLINICAL OBSERVATIONS AND IMMUNOBIOLOGICAL ASPECTS

On the basis of the present-day investigations in anatomy and physiology it may be concluded that the lymphatic system of the neck is part of the general lymphatic defence system of the organism which, due to its localization in the anatomical sense, holds a specific position for the organs of the head and the neck.

While the entire lymphatic system of the other parts of the body is directed towards the thoracic duct and the right lymphatic duct, the lymph from the region of the head and the neck flows towards the supraclavicular fossae and it is therefore clear why, in contrast to the other localizations, metastases in the region of the neck remain in this site for a longer period before passing into the region of the mediastinum or into other organs as distant metastases.

The spreading of the tumour from the primary focus into the lymphatic nodes is embolic in character, thus between the tumour and the metastases there is no continuity of malignantly altered lymphatic ducts. It was also realized that the tumour does not spread from one lymph node to the other but unifocal or multifocal spreading from the primary lesion is present from the very beginning. Multifocal spreading marks a greater discord

between the tumour and the organism. Our clinical experiences have taught us that these forms are highly malignant. It is therefore necessary to undertake a histological examination of all removed lymph nodes in order to avoid radical resection.

Siegel & Fisch (1965) showed that following radical resection of the neck the lymphatic system via the subcutaneous and the lymphatic nodes on the same and the contralateral side. Welsh & Welsh (1963) and Press & Press (1960) showed that following block resection of the neck, contralateral lymphatic nodes usually appear. In 1947 Furuta showed the regeneration of the lymphatic system whereby an important role was played by the youth of the experimental animal. In histological investigations (Krayina et al 1969) in cases of radical neck resection the fatty and fluffy tissue of the neck stretching from the subcutaneous to the deep musculature of the neck, small islands of lymphoreticular elements are present, which can subsequently develop into smaller or larger lymph nodes. The lymphatic nodes which develop after radical resection are frequently located subcutaneously in the deep structures of the neck, leading to complications.

The following experiments also show the role of the lymphatic apparatus in the development of malignant tumours.

Cnife (1969) studied the relationship between the tumour and the regional lymphatic nodes. He implanted S-180 into the heel of a rat and observed the course of the disease after removal or irradiation of the lymphatic nodes in the popliteal region. If these nodes were extirpated within a period of 4 to 10 days after implantation of the tumour, not only was there reduced resistance against the tumour but a higher incidence of pulmonary metastases developed too. Extirpation of the lymphatic nodes 10 days after implantation did not confirm this dependence. Besides he also obtained similar results with ordinary mastectomies in the first stage of a tumour.

than by radical resection of the  
and the regional lymph nodes  
Clinical experiences might serve to cor-  
these results of Crile's investigations  
the development of regional  
uses after two or more years and with-  
recurrence would indicate the defen-  
of the regional lymph nodes. The  
of enlarged lymph nodes, histologi-  
negative, on the side of the primary  
moreover indicates the participation of  
apparatus in the defence against the  
ant process. A more marked lympho-  
reaction in the environs of the tumour is  
ive of a better outcome of the malignant

It is established without any doubt  
lymph nodes take part in the forming  
of immunoglobulin elements via their  
elements and by means of the  
lymphocytes in the creation of sessile and  
of cellular antibodies which play an  
important role in the development of the late  
reaction. While in malignant diseases  
antibodies maintain their high  
level for a long period, with the develop-  
of the disease the lymphocytic reaction  
disappears rapidly. Thus we could establish that  
in 7% of our patients a decrease in the  
number of lymphocytes in the blood below  
was a sign of the fatal outcome of the

15 years ago we established that in  
patients suffering from malignant tumours in  
the RL region the quantity of immunoglobu-  
lins showed an increase, though this higher  
level did not indicate a less malignant lesion,  
on the contrary indicated the increased  
strain on the organism and that only in the  
late stage the immunoglobulin quotient  
tended to decrease somewhat.

Over the last few years it was moreover  
found that malignant tumours develop more  
frequently after transplantation of organs be-  
cause of the application of immunodepressant  
drugs and since then immunology has begun to  
penetrate more and more into the problems of

malignant disease. Eilber & Morton (1970)  
showed that in 93% of all their patients with a  
late negative reaction to DCNB (dinitrochloro-  
benzene) the prognosis was poor. Negative  
tuberculin reaction also appears in the major-  
ity of late cases. The organism reacts to  
foreign substances through the lymphocytes,  
from which such notions are then transmitted  
to the next few generations. The antigen  
capacity of tumours has so far been noted  
especially in malignant melanoma, osteogenic  
sarcoma and Burkitt's lymphoma. Testing at  
our clinic the capacity of the organism to react  
to its own tumour in the same way as to a  
foreign antigen, we were unable to observe  
any legality between this reaction and the  
prognosis of the disease. On the contrary we  
were able to establish a very small number of  
positive reactions. The observation, however,  
that in 90% of all patients with very malignant  
tumours the number of lymphocytes in the  
blood falls below 10% is no doubt a very  
significant symptom in the prognosis, and at  
the same time an indicator of the general im-  
munobiological state in combating this  
malignant disease.

All these examples and clinical observations  
confirm that the lymphopoietic apparatus is  
not a passive track alone over which the  
malignant cells slide, but a defensive ap-  
paratus by means of which the general defen-  
sive power of the organism against the  
malignant process is expressed.

## CLINICAL MATERIAL

For the study of the problem of unilateral and  
bilateral resection of the neck we selected the  
clinical material covering the period 1960 to  
1970 but only of those patients in whom classic  
complete unilateral or bilateral neck resection  
had been performed. We considered that for  
this problem chosen for the subject of our  
study it might be sufficient to take into consid-  
eration carcinoma of the larynx, hypopharynx  
and the skin. While in cases of laryngeal



Table I *Radical neck dissection performed in 108 cases of laryngeal cancer (1960-70)*

RP=recurrence of the primary tumour

RM=recurrence of the metastases

Distant metastases lung 3 cases

	Living	Dead	Unknown	Total
<i>Unilateral neck dissection</i>				
T <sub>1</sub> N <sub>1</sub>	2	1 RM 1	2	5
T <sub>2</sub> N <sub>1</sub>	13 RP 1 RM 1	5 RP 1 RM 4	3	21
T <sub>3</sub> N <sub>1</sub>	20 RM 2	28 RP 10 RM 24	7	55
T <sub>4</sub> N <sub>1</sub>	-	3 RP 1 RM 2	1	4
	35	37	13	85
<i>Bilateral neck dissection</i>				
T <sub>1</sub> N <sub>2</sub>	-	-	1	1
T <sub>2</sub> N <sub>2</sub>	-	-	-	-
T <sub>3</sub> N <sub>2</sub>	3 RM 1	10 RP 3 RM 8	2	15
T <sub>3</sub> N <sub>2</sub>		1 RP 1 RM 1	-	1
T <sub>4</sub> N <sub>2</sub>		5 RP 2 RM 5	-	5
T <sub>4</sub> N <sub>2</sub>	-	1 RM 1	-	1
	3	17	3	23

carcinoma we kept in that period to the principle of carrying out therapeutical block resection only in cases of carcinoma of the hypopharynx we have been performing preventive block resection of the neck and extirpation of the primary tumour in the same act. The lymph nodes of the neck extirpated because of carcinoma of the larynx were histologically positive in all our patients and in carcinoma of the hypopharynx negative in one case only.

I In laryngeal carcinoma (Table I) we achieved recovery in 35 of 85 patients (41%) by means of unilateral neck resection while in cases where bilateral resection had to be applied this ratio was 3/23 thus amounting to a

mere 13%. A separate analysis of tumours in group T<sub>1</sub> and T<sub>2</sub> showed percentage of recoveries in case primary tumours was higher (15/57%). The analysis of the survivors with a primary tumour showed a recurrence of the primary only one of our 38 cases while developed in 4 cases out of these 3 was significantly changed in the deaths where the primary tumour 18 of 54 cases while regional showed recurrence in 46 out of

II In carcinoma of the larynx pharynx (Table II) or in carcinoma of the hypopharynx (Table III) the percentage of recoveries was considerably low. Patients with unilateral metastases (24%) survived while of the 16 patients with bilateral metastases none survived. Patients with this localization of the tumour who survived, there was 1 case of recurrence of the primary and 1 case of recurrence of the metastases. These results are in an

Table II *Radical neck dissection performed in 23 cases of laryngeal and hypopharyngeal cancer*

Distant metastases lung 1 case

	Living	Dead	Unknown
<i>Unilateral neck dissection</i>			
T <sub>1</sub> N <sub>0</sub>		1	-
T <sub>1</sub> N <sub>1</sub>	3	6 RP 3 RM 5	1
T <sub>2</sub> N <sub>1</sub>	1	-	-
	4	7	1
<i>Bilateral neck dissection</i>			
T <sub>2</sub> N <sub>2</sub>		8 RP 3 RM 7	-
T <sub>3</sub> N <sub>2</sub>		3 RP 1 RM 3	-
		11	-

### III Radical neck dissection performed in cases of hypopharyngeal cancer

11 cases lung 5 cases vertebral column 1

	Living	Dead	Unknown	Total
<i>Unilateral neck dissection</i>				
-	-	1	1	
2	-	2	4	
1	2	1	4	
	RP, 1			
	RM 2			
-	3	-	3	
	RP 1			
	RM, 3			
-	5	-	5	
	RP, 3			
	RM 5			
3	10	4	17	
<i>Bilateral neck dissection</i>				
-	1	-	1	
	RM 1			
-	4	-	4	
	RP 2			
	RM 4			
-	5	-	5	

to the results in the group of deaths in which recurrence of the primary tumour was in 14 of 33 patients and metastases in 30 patients. Distant metastases developed in hypopharyngeal carcinoma in 3 of 108 patients (3%) and in 7 of 45 patients (16%) in carcinoma of the hypopharynx.

Our analysis included carcinomas of the head and the neck which were primarily treated by irradiation and usually had a somewhat better prognosis than the other localizations of carcinoma of the head and the neck. Of the 12 patients, however, in whom regional metastases had developed, 11 were excluded from this group: two carcinomas which *a priori* are highly malignant and regional metastases develop, this high lethal rate in carcinoma of the skin of the head and the neck with regional metastases clearly shows that the appearance of metastases is a sign of weakening of the immunological forces of the organism.

### DISCUSSION

We believe that on the basis of our clinical studies which particularly deal with carcinoma of the larynx, hypopharynx and the skin of the head and the neck, we may draw some conclusions about the value of resection in general, about the relationship between bilateral and unilateral resection of the neck and of the value of prophylactic resection.

Although with regard to block resection our attitude in carcinoma of the larynx is generally therapeutic and carcinoma of the hypopharynx prophylactic, the final results clearly indicate that the malignancy of the tumour and the relationship of the lymphatic system towards the degree of malignancy are of decisive importance for the outcome of the disease. Thus, for instance, laryngeal carcinoma even subsequent block resection of a smaller tumour gives good results, while in carcinoma of the hypopharynx in spite of the prophylactic resection of the neck the results are markedly poor. Naturally it is wrong to speak of prophylactic block resection in carcinoma of the hypopharynx if, in spite of the negative palpatory finding, the presence of metastases is proved histologically.

Invasiveness of carcinoma of the hypopharynx *vis-à-vis* carcinoma localized in the hypopharynx and the larynx, leads much more rapidly to destruction of the defensive powers of the organism, just the same as the deeper position of the metastases in such a primary localization leads to infiltration of the vagus nerve and the carotid artery. Not a single one of our patients with infiltration of the vagus nerve or the carotid survived. Carcinoma of the skin was taken as an example of a tumour which metastasizes. However, the appearance of metastases in carcinoma of the skin indicates marked invasiveness of the tumour and a weakening of the immunobiological forces of the organism in the struggle against the malignant lesion. The following conclusions are derived from these considerations:

We are not in favour of prophylactic

resection, since this would be contrary to our knowledge of the role played by the immunobiological factors in the struggle against the malignant lesion

Evacuation of the neck must be performed in carcinoma of the larynx  $T_3$  and in hypopharyngeal carcinoma because histologically positive lymph nodes are usually found in this site in large numbers

The great hope expressed by Crile in 1906 that surgical therapy would solve the problem of carcinoma of the head and the neck has so far not come true. The high percentage of recurrences after unilateral and especially after bilateral resection of the neck indicates that other therapeutic means must also be applied in the struggle against malignant growths in the head and the neck

Radical neck resection has been performed in 270 patients—unilateral in 209 and bilateral in 61 of our patients. Of the 209 patients in whom unilateral resection was performed 78 (37%) survived, while of the 61 patients in whom bilateral resection had to be performed only 3 (4.9%) survived

The organism engages in the struggle against the malignant lesion with its immunobiological apparatus. The first defeats of the organism in this struggle are reflected by the appearance of unilateral metastases, are continued by the development of bilateral metastases, and finally the organism and its defence mechanisms break down completely with the appearance of distant metastases. Compensation and recovery may occur in unilateral metastases, much less frequently in bilateral and only exceptionally in cases where distant metastases have developed

## ZUSAMMENFASSUNG

An der Hals Nasen Ohren Klinik der Medizinischen Fakultät in Zagreb wurden von 1960 bis 1970 270 unilaterale und bilaterale Halsresektionen bei verschiedenen

primären lokal-extendierenden Tumoren durchgeführt. Auf Grund dieser Analyse und der immunologischen Betrachtungen folgen die Autoren eine prophylaktische Blockresektion wird nicht befürwortet, weil Erkenntnissen von der Rolle der immunologischen Faktoren im Kampf mit der malignen Läsion. Bei Larynxkarzinom  $T_3$  wie auch bei hypopharyngeal Karzinom soll die Halsresektion durchgeführt werden weil hier bei einem grossen Prozentsatz die Lymphknoten histologisch positiv sind.

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# INHIBITORY PROCESSES IN THE MEDIAL GENICULATE BODY

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Inhibition in the medial geniculate body was studied with a double click technique. A conditioning click evoked a marked inhibition of field potentials and unit responses to a subsequent test click. Cyclic inhibition with a period of about 150 ms was seen in the medial geniculate body and the auditory cortex, but not in the inferior colliculus. The postsynaptic excitability was studied by recording from the killed ends of the thalamo-geniculate fibres. A preceding click reduced the direct response to a test stimulus delivered to the medial geniculate body indicating the presence of postsynaptic inhibition. The excitability of the fibres from the inferior colliculus terminating in the medial geniculate body was studied with a modified Wall (1958) method. The results indicate the presence of a presynaptic inhibitory mechanism in the medial geniculate body. The techniques used do not allow an estimation of the relative contribution of the two inhibitory mechanisms to the inhibition at the medial geniculate level.

Auditory nerve fibres respond to loud sounds over a large frequency range. With intensities just above threshold, however, they respond to a distinct frequency. Plotting of the response threshold versus frequency will, therefore, give a wide angled tuning curve. The frequency of the tuned peak of the tuning curve represents the characteristic frequency (CF) of the fibre (Kiang et al 1954, Kiang et al 1965). At higher intensities of the auditory nervous system the tuning curves are progressively narrower, and are reported to be most contracted at the

medial geniculate (MG) level (Katsuki et al 1959a). The explanation for this phenomenon may be sought in part by the prominent inhibitory processes found at various levels in the auditory pathways. Thus, Whitfield (1955), recording from units in the trapezoid body, found a distinct reduction of the response to the second of two tones compared with the response to a single tone of the same frequency and loudness. Similarly, Katsuki et al (1959b) found that spontaneous as well as tone evoked discharges of single units in the higher levels of the auditory pathway were suppressed when a background tone was delivered simultaneously with the test signal. The degree of the suppression was dependent upon the level of the background sound or noise. Refining these observations Nomoto et al (1964) found that the most effective reduction was produced by a conditioning tone having a frequency either just above or just below that of the test tone.

Atkin et al (1966) have shown that spontaneously active units in the medial geniculate body are suppressed for 40 to 200 ms after a click. In their work the possibility of both pre- and postsynaptic inhibition in the MG, modulating the responses to sound stimuli was discussed, but not tested. However, postsynaptic potentials (IPSPs) have been recorded from MG neurones (Nelson &

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Erulkar, 1963) Excitability studies following stimulation of the brachium of the inferior colliculus have demonstrated long lasting inhibition, however, without distinguishing between pre- and postsynaptic inhibitory mechanisms (Aitkin & Dunlop, 1969) In view of the presence of both pre- and postsynaptic inhibition in other parts of thalamus (Andersen et al., 1964, Burke & Sefton, 1966), a similar arrangement in MG seems possible

The aim of the present investigation has been to study the time course and degree of the click-induced inhibition found in MG and to study the mechanisms underlying the inhibition In particular, the possibility that presynaptic inhibitory mechanisms could be present in addition to the earlier known postsynaptic inhibition, was tested It will be shown that presynaptic depolarization, which is associated with inhibitory processes in other parts of the central nervous system (Eccles, 1969) operates in MG

## METHODS

Adult cats were anaesthetized with 30 mg sodium pentobarbital per kg, given intraperitoneally Maintenance doses (10 mg/kg) were given intravenously to maintain a moderate light barbiturate anaesthesia, characterized by a withdrawal of the foreleg on pinching of the paw The head was fixed in a head holder with ear bars The bar to the test ear was hollow In some animals with wide bony auditory canals the bars had to be pushed so far in to fix the head that the ear drums were damaged In such animals the sound was increased by 15–20 dB but the shape of the input/output curve recorded from the medial geniculate was similar to that normally observed

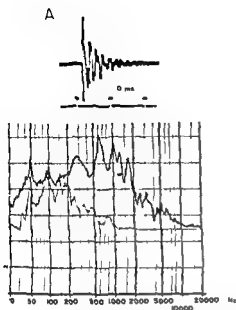
The skull overlying the dorsal part of the brain was removed and the skin flaps were sewn to a ring to prepare a pool which was filled with warm liquid paraffin The dorsal part of the thalamus was exposed by removing the overlying neocortical tissue of the lateral and suprasylvian gyri and the hippocampus

The MG body is situated only 6–9 mm from the dorsal surface of the thalamus. It is difficult to approach the surface of the inferior colliculus could be seen directly The exposed area was marked to ensure accurate placement of the recording electrodes By comparing the results with and without cortical removal no significant change of the responses could be noted after the tests applied

Extracellular recordings of unit action potentials were made by glass capillaries filled with solutions of 4 M NaCl or 3 M KCl. Micro electrode signals were fed to a pre-amplifier and displayed on an oscilloscope with a 100-fold amplification To counteract variations in the responses, particularly with regard to the tendency of rhythmic activity in the thalamus, a computer of average transients was used as part of the study

Surface records from the auditory cortex were made with small platinum ball electrodes (0.5 mm diameter) Recordings from the "killed ends" of the thalamo-cortical tract were made from the white matter under the primary auditory cortex by using a thin platinum wire insulated with polyethylene except at the tip and cut off sharply After the cortex had been sucked away, the electrode was moved over the surface of the white matter in order to find the best recording point Larger signals were obtained by pushing the electrode approximately 1 mm into the white matter When properly placed, the "killed ends" recording appears as a positive monophasic deflection Care was taken to remove all surrounding auditory cortex to avoid possible contamination with cortical field potentials The signals from the white matter or the auditory cortex were amplified and displayed on an oscilloscope

Clicks were elicited by feeding square wave pulses of 50  $\mu$ s duration to an amplifier which drove a loudspeaker Clicks were preferred to tones because excitability studies require a brief stimulus with abrupt onset The click had a sharp initial transient which evoked a recognizable response at all levels



(A) Recording of a click of 108 dB (B) Frequency spectrum of the same click (heavy line) analysed in 3 dB frequency bands. Thin line gives the background noise of the experimental room. The amplitude is sound pressure in  $10^{-9}$  dyn/cm<sup>2</sup>.

auditory nervous system (Rosenzweig & Nath 1953). It was delivered by the speaker to a funnel feeding a tube. The end of the tube from the loudspeaker to the bar inserted into the external auditory was 56 cm and had a bore of 7 mm. The bar was connected to a Y shaped air pipe, a hollow bar attached to each end. The bars were 10 cm long with a bore of 3 mm. One bar was inserted into the external auditory ear, whereas the other was connected to an artificial ear consisting of a rubber tube opposite a linear condenser microphone. The cavity was lined with soft material to imitate the soft lining of the external and middle ear and its volume measured 1.3 cm<sup>3</sup>. As measured in the artificial ear the shape of a click with an amplitude of 108 dB is shown in Fig. 1A. The oscillations are heavily damped with an interpeak period of approximately 1.7 ms corresponding to a frequency around 600 Hz. The amplitude of the second wave usually

measured less than 60% of the first. The frequency content of the click is given in Fig. 1B (heavy line) showing a large peak at 620 Hz with smaller peaks at 280, 1000, 1240, 1660 and 2200 Hz. After subtraction of the background noise (thin line) the 620 Hz peak had the largest amplitude throughout the intensity range used in these experiments. With the soft linings of the external auditory canal and the middle ear the attenuation of the higher frequencies is probably similar to those observed in the artificial ear employed. Thus the frequencies above 1000 Hz have contributed relatively little to the results obtained particularly at the higher click intensities.

The microphone output was calibrated in free field in a camera silentia. A calibrated generator delivered a continuous tone of 1000 Hz. When the tone had an intensity of 74 dB (re  $0.0002$  dyn/cm<sup>2</sup>) the microphone signal gave an output of 1 mV. Other intensities were calculated by help of the following equation

$$p = 74 \text{ dB} + \log \frac{p_2}{p_1} \text{ where } p_1 \text{ is 1 mV and } p_2 \text{ the peak}$$

amplitude of the microphone signal in mV.

The microphone had a linear characteristic for all frequencies of the click employed.

## RESULTS

### Pattern and time course of MG inhibition

A conditioning click caused a long lasting inhibition of the response of MG cells to a subsequent test click.

In Fig. 2A the upper trace shows the field potential of MG in response to a conditioning (C) and a test (T) click. The responses consist of a negative wave (N wave) with superimposed spike followed by a positive wave (P wave). The second trace shows the click as recorded by the microphone. The intensified portions of these two sweeps with the test response (T) are expanded and shown in the third and fourth trace. In B, C and D are shown similarly expanded responses to the

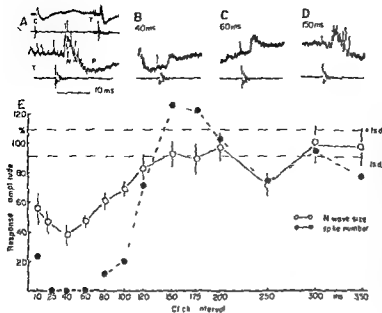


Fig. 2 Time course of inhibition of medial geniculate body. (A) Response of medial geniculate neurones (upper) to a click (120 dB) (second trace); intensified portions of these two traces expanded in the two lower traces. As lower two traces in (A) by response was preceded by a conditioning click (122 dB) at the indicated interval. The size of the medial geniculate (O) and the number of spikes (●) against the interval between the conditioning and test clicks.

same test click as they appear when preceded by a conditioning click at the indicated intervals. The reduction consists of an abolition of discharges and a diminution of the *N*-wave (B, C). On the other hand, at 150 ms interval (D) the *N*-wave was normal, but the number of spikes was larger than normal. Such post-inhibitory rebound responses were often present, but varied in size and exact timing from one trial to the next. The amplitude of such post-inhibitory rebound was, therefore, calculated from the number of trials (E). After the rebound, there was a new period with reduced responses giving a dip in the inhibitory curve at an interval of 250 ms.

Two significant features of the MG inhibitory curve distinguishes it from the inhibition measured at the inferior collicular (IC) level. First, the reduction of the *N*-wave of the medial geniculate was more pronounced than the effect on the corresponding *N*-wave of the inferior colliculus. Second, the MG inhibitory curve shows a cyclic behaviour contrasting the single-peaked inhibitory curve from IC. When measured as the probability of spike discharge the difference between the depression observed at the inferior collicular and medial geniculate levels became even more evident.

#### Relative contribution of inhibition at inferior collicular and medial geniculate level

In order to obtain reliable results on the relative contribution to inhibition of different relay stations, such comparisons were made using results obtained from individual animals. Unfortunately, the size of the potentials does not necessarily bear a relationship to the input sound pressure. However, over a range from threshold to saturation the inferior collicular and the *N*-waves and the cortical surface post-inhibitory potential showed a remarkably similar development having near identical threshold and saturation points. The last point is certain than the threshold value because of the relatively large contribution of several potentials may contribute relatively little to the total potential because of the increased size of the synaptic potentials and unit discharges which comprise the field potential. In this restriction a relative indication of the contribution to the inhibition at various levels was gained by referring the depression of an input/output curve of the field potential that nucleus particularly when operating in the range given by the lower two-thirds

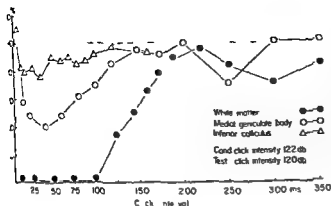


Fig 3 Effect of a conditioning click (122 dB) on subsequent responses to a test click (120 dB) recorded from the auditory radiation (positive killed end response ●) the medial geniculate body (N wave ○) and the inferior colliculus (N wave △) All responses are expressed as per cent of the control response (ordinate) in relation to the interval between the conditioning and test clicks (abscissa)

Since the active range of the sound sources that is from the threshold to the saturation point was located at somewhat different positions on the intensity scale in various animals only the difference in dB was used. In this way each experiment ended as its own control.

Measured as a percentage reduction of the responses relative to the control level the inhibitory efficiency of the inhibitory processes at various stations in the auditory system appeared to differ. Fig 3 gives the results from series of experiments performed on the same animal. A conditioning click with an intensity of 122 dB produced a depression of the responses to a test click of 120 dB at both the inferior collicular, medial geniculate and auditory radial level. The size of the N wave recorded from the inferior collicular level is given as open triangles whereas the amplitude of the medial geniculate N wave is given as filled circles. The symbols give the size of the response as a function of the interval from the preceding conditioning click. Both the IC and the MG N waves are predominantly due to EPSPs spikes contributing only a small part as seen during rapid tetanic stimulation. The reduction of the N wave was larger in the medial geniculate than in the inferior collicular record. However a significant later change occurred in the record from the white matter which indicates a reduction of the number of discharging MG cells. At intervals

from 20 to 75 ms between the conditioning and test clicks there was total abolition of the discharge of MG cells. After 200 ms the response was back to normal. At longer intervals a new reduction of the discharge occurred indicating cyclic inhibition. The cyclic type of inhibition was not seen below the MG level and corresponds to the pauses between the repetitive burst discharges of MG units in

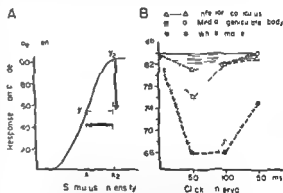


Fig 4 Relative contribution of various stations of the auditory nervous system to inhibition of click-evoked responses (A) Heavy line gives size of response (ordinate) as a function of stimulus intensity (abscissa). A response reduction from  $Y_2$  to  $Y_1$  (vertical arrow) corresponds to a reduction of the stimulus intensity from  $X_2$  to  $X_1$  (horizontal arrow). (B) Using the method sketched in (A) the inhibitory curves obtained from the inferior colliculus (△) the medial geniculate body (○) and the white matter (●). The inhibition produced by cells caudal to the inferior colliculus is represented by the horizontally striped area, the inhibition added at the inferior collicular level by the obliquely hatched area and the inhibition added at the medial geniculate level by the dotted area.



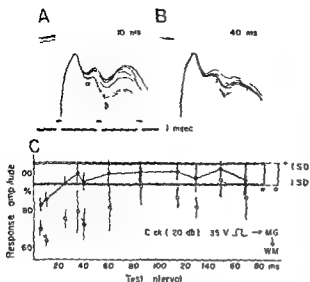


Fig. 5. Test for postsynaptic excitability of MG neurones. A: The  $\alpha$  deflection recorded from the auditory radiation indicates the number of postsynaptic elements excited directly by a stimulating micro-electrode in MG. The  $\beta$  deflection indicates the number of cells excited transsynaptically by activation of intranuclear presynaptic fibres. The supplied lines are tracings of superimposed control responses. Superimposed on these records are 5 other records (full lines) in which the test stimulus was preceded by a click of 120 dB at intervals of 10 ms (B) As (A) but with a click interval of 40 ms (C) Time course of inhibition of  $\alpha$  (●) and  $\beta$  deflection (○) as a function of interval between conditioning (122 dB) and test stimulus of 35 V (abscissa). Vertical range bars give  $\pm 1$  standard deviation.

response to a click (Atkin & Dunlop 1969; Etholm 1975).

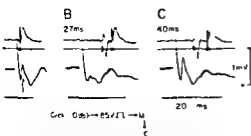
Another way to measure the relative contribution of the inhibition at various levels is to compare the inhibitory effect with the input/output curve for each recording station obtained at the start of the experiment. The relative reduction is calculated using the technique shown in Fig. 4A. The input/output curve is drawn with a full line. An unconditioned test stimulus of an intensity  $X_2$  produced a response of amplitude  $Y_2$ . When preceded by a conditioning stimulus, however, the amplitude was reduced to  $Y_1$  (vertical arrow). The observed inhibition is equivalent to a reduction of the input sound pressure from  $X_2$  to  $X_1$  (horizontal arrow). Using this technique to compare the inhibitory curves obtained from the inferior collicular medial

geniculate and white matter with their respective input/output curves in a single experiment, the graph in B was obtained. Since the size for its greatest part was dependent on the size of the afferent volley, the horizontally hatched area mainly represents the inhibition generated below the inferior colliculus. The obliquely hatched area refers to the size of the medial geniculate. It was mostly independent upon the number of collicular fibres discharging. There is a short-lasting inhibition seen here, which is a result of the contribution of inhibition at the collicular level. The large dotted area, however, was measured from the white matter and indicates the reduction of medial geniculate neuronal discharge. This area indicates, therefore, inhibition added at this level. None of these measurements are related to the inhibitory accretion. It is only a relative indication of the size of the present.

### Postsynaptic inhibition

In accordance with the experiences of & Erulkar (1963), penetration of the geniculate neurones proved relatively difficult. In some cells, however, it was possible to record intracellularly for a short period. The membrane potential was always low (around 40 mV), most likely due to damage to the cell membrane. The click was often eliciting a depolarizing wave (EPSP) which there were one or two local or abortive spikes followed by a brief hyperpolarization (50–60 ms) interpreted as an IPSP because it corresponded to the spontaneous discharges. Often this was followed by a second IPSP after a single click was given.

Another test for postsynaptic inhibition can be made with the technique used by Renshaw (1940). In the spinal cord, a stimulating electrode inside the medulla will excite postsynaptic elements as well as presynaptic fibres. Recorded from the auditory radiation, the excitation of



Excitability tests of presynaptic fibres terminating in the medial geniculate body. (A) Response of the inferior olive (upper trace) in response to an 85 V pulse (red) through a tungsten micro-electrode located in the medial geniculate nucleus. The intensified  $\alpha$  of the upper trace is expanded in the third trace. It shows a negative spike signalling the antidromic action of inferior collicular neurones (arrow). (B) As in (A) but the test pulse was preceded by a click as used by the microphone record (second trace). The interval between the click and test pulse was 27 ms. The  $\alpha$  spike was markedly reduced. (C) As in (B) but the click test stimulus interval of 40 ms. The size of the  $\alpha$  deflection was increased above normal. This indicates that the constant test pulse to the medial geniculate body excited more fibres than in the control condition in (A). The increased antidromic field potential is a measure of increased excitability of fibres of the olivary inferior colliculus terminating in the medial geniculate body.

aptic elements will be picked up as a short latency monophasic positive deflection. This is exemplified by the  $\alpha$  deflection in Fig. 5A. Stimulation of intranuclear presynaptic terminals will, after the synaptic delay, also produce a discharge of the MG cells provided not all are refractory following a too strong test stimulation. This will lead to a second deflection in radiation record, labelled  $\beta$ .

When a click changes the excitability of the olivary elements of the medial geniculate body, a subsequent test stimulus will produce a response which differs from the control response. If the postsynaptic elements are hyperpolarized the direct stimulus will excite fewer neurones. In the present experiments this was indeed observed in the form of a reduced  $\alpha$  deflection (Fig. 5A). In addition the  $\beta$  deflection was reduced (Fig. 5B). The latter observation could be due to either postsynaptic or presynaptic inhibition, or to both, due to a certain spontaneous

excitability associated with the spontaneous barbiturate spindles, a series of records was measured at each interval in order to minimize any alterations other than those produced by the click itself. In Fig. 5A and B are shown 5 superimposed control responses to stimulation within the medial geniculate body with a constant test stimulus (stippled lines) traced on top of each other. Superimposed on these records are 5 records in which the test stimulus was preceded by a 120 dB click (full lines), the interval being 10 ms in A and 40 ms in B. There was a small, but significant decrease of the  $\alpha$  deflection when the click interval was shorter than 30 ms. A somewhat greater reduction occurred in the  $\beta$  deflection. However, this deflection may be due to either postsynaptic or presynaptic inhibitory mechanisms or to a combination of the two. When the click test interval was increased to 40 ms, no definite reduction of the  $\alpha$  deflection could be measured, whereas a small reduction of the  $\beta$  deflection was still present. The time courses of the reduction of the two deflections are seen in C. As expected from the situation in other thalamic nuclei, the duration of the  $\alpha$  wave depression was considerably shorter than that of the  $\beta$  deflection (Andersen et al., 1964). This indicates that the synaptic excitation is a more sensitive test for cell excitability than the directly stimulating electrical current.

The relative depression of the  $\alpha$  and  $\beta$  deflections was dependent upon the test strength. If the latter was too strong, no depression was seen at all in the  $\alpha$  deflection, possibly due to current spread to the neighbouring Ranvier nodes, thus escaping the hyperpolarization which most probably took place at the cell body (Maekawa & Purpura, 1967). The time course of the depression of the deflection roughly coincided with the depression of spike discharge and reduction of the N-wave.

Similar reductions in the  $\alpha$  and  $\beta$  deflection were also seen when the test shock was delivered in the trough of spontaneous

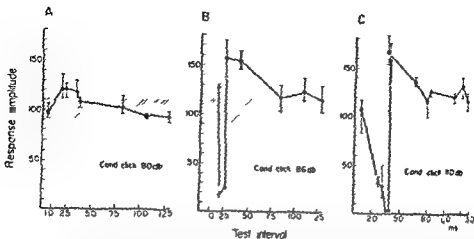


Fig 7 Time course of the excitability changes in fibres from the inferior colliculus terminating in the medial geniculate body (A) A conditioning click of 80 dB caused a weak increase of afferent fibre excitability measured as size of antidromic spike recorded from IC (B) More pronounced effect by a 86 dB conditioning click except at

short intervals where there was a sudden re-act antidromic field potential signalling blocked on large number of cells (C) A conditioning click gave an even more pronounced initial re-act long-lasting increase of brachium fibre excitability

spindle activity of the MG cells. In such cases, however, the degree of the inhibition was smaller than that following a strong click.

In conclusion, testing the postsynaptic excitability of MG cells with the Renshaw technique (1940) gave evidence that part of the inhibition observed in the medial geniculate body involves postsynaptic inhibition.

#### Presynaptic inhibition

In order to test for presynaptic inhibition, a modification of the original Wall (1958) technique was used. A major part of the orthodromic afferent impulses to the medial geniculate body comes from the ipsilateral inferior colliculus. Thus by stimulation within the medial geniculate body terminal fibres of the brachium of the inferior colliculus should be excited, giving rise to antidromic invasion of inferior collicular neurones. This was found to be the case. In Fig 6 the upper trace shows a record of a micro electrode in the inferior colliculus and the second trace gives the click response. The intensified part of the inferior collicular response is expanded and shown in the lower trace. Following the stimulus artefact, there was a short negative deflection with a latency of about 0.9 ms (Fig 6A, arrow)

The amplitude of the deflection was 2 mV in the centre of the inferior colliculus and fell quickly outside its border. Furthermore, with high impedance electrode deflection was seen to be composed of discharges having short and constant latencies.

Therefore, this deflection is interpreted as due to antidromic invasion of a number of inferior collicular cells.

Because a series of linearly increasing stimuli delivered through the medial geniculate stimulating electrode gave gradually increasing antidromic responses, the size of antidromic inferior collicular response was taken as an indicator of the number of brachium inferior collicular terminals which was excited.

A conditioning click produced a large response in the inferior colliculus (Fig 7 upper trace). In the test response, delivered 20 ms later there was a near total reduction of the antidromic response. However, when the click test interval was increased to 80 ms the antidromic test potential was not reduced below the control record, implying a larger number of presynaptic terminals in the medial geniculate body had been stimulated.

The time course of the excitability changes

free different strengths of conditioning are given in Fig. 7. With relatively weak conditioning clicks (80 dB) the excitability of trachium inferior collicular terminals in iG showed an increase with a maximum click test interval around 25 ms. The effect was relatively weak and lasted only 40 ms. On increasing the conditioning click to 110 dB however the picture changed. The excitability rise was more prominent showing a maximal amplitude increase of more than 100% and a total duration of more than 100 ms. However at intervals between 20 to 27 ms there was a nearly complete abolition of the antidromic test response. This marked reduction of the antidromic response at intervals around 25 ms was a constant feature with all conditioning clicks. With a 110 dB conditioning click for example the duration of the block increased somewhat so that it lasted until 35 ms. After that time however the antidromic response was markedly enhanced again indicating an increase of the excitability of the fibres terminating in the medial geniculate body.

## DISCUSSION

### Parameters

In excitability tests like those used in the present investigation it is important that the test stimulus was delivered as abruptly as possible. The time course of the click used in the present study (Fig. 1A) fulfils this criterion. The initial deflection started suddenly and was much larger than the subsequent deflections and was part of the main frequency peak at 620 Hz. With increasing strength of the clicks the amplitude above background noise of the major frequency components increased markedly. At the highest click intensities used there was a small reduction of the amplitude of two of the highest frequency peaks, 1240 and 2200 Hz. Thus with increasing click strength there was no increase of any particular frequency which could account for a systematically different

conditioning situation than that used at lower stimulus strength. The low background noise (around 30 dB) at the main frequency of 620 Hz was acceptable for the tests used in the present investigation.

### Duration and relative size of inhibition at inferior collicular, geniculate and cortical level

A main feature of the inhibitory curves obtained at the various stations of the auditory system was their large difference in duration. At the inferior collicular level the maximal inhibition did not last beyond 50 ms whereas the inhibition at the geniculate and cortical level lasted for about 120–150 ms. Furthermore at the two last levels the inhibition was clearly cyclic, corresponding to the repeated P waves that are seen in response to a single click at appropriate levels of barbiturate anaesthesia. A probable explanation is that the medial geniculate body contains an inhibitory mechanism that is different from that operating at lower levels of the auditory system.

A second feature of the inhibition found at various auditory stations is its effectiveness or size. In order to measure the relative size of the inhibition at the different levels it was necessary to measure this against an input/output curve for each recording station. A prerequisite for using the input/output curves to measure the relative inhibition at various auditory stations is that the threshold is the same, that the increase shows a similar pattern at all levels to be compared. From the threshold to 20–25 dB above threshold this was found to be the case. Within this range of the input/output curves it was obvious that the inhibition occurring at the medial geniculate level added greatly to the total inhibition in the auditory system.

The MG inhibition is probably of the surround type, being largely responsible for the very narrow tuning curves obtained at the medial geniculate level (Katsuki et al. 1959a). The cyclic nature of the inhibition is exemplified by the post stimulus histogram.

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## COCHLEAR INNERVATION IN THE GUINEA PIG

### II The Spiral Tunnel Bundle

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The organization of nerve fibers in the spiral bundle (STB) of the guinea pig organ of Corti has been studied by light and electron microscopy. In the STB fibers were found to run toward the base or about equal numbers while in the upper turns they predominantly in an apical direction. Long spiral fibers of 100 microns or more in length were abundant in the third and fourth turns but were rarely seen in the lower turns. No synaptic contacts between tunnel bundle fibers could be found and no afferent nerve fibers were found in the bundle. Furthermore, interruption of the nerve supply resulted in complete degeneration of the tunnel bundle. The tunnel bundle does not, therefore, appear to be a site of synaptic interaction between afferent and efferent elements.

On the basis of electron microscopic observations of material from the cat, Spoendlin (1969) concluded that over 90% of all cochlear efferent nerve fibers terminate on the inner hair cells. In addition, a recent quantitative study of the nerve supply of the guinea pig organ of Corti by Morrison et al (1975) provided evidence that 85-90% of the afferent fibers innervate the inner hair cells. Spoendlin's work on efferent innervation of the cat has stimulated increasing interest in the role of neural elements near the inner hair cells in information processing at the periphery (Lynn & Liberman, 1970; Nieder, 1971; Zwislocki & Liberman, 1973).

The idea that interaction between the various types of nerve fibers in the vicinity of the

inner hair cells is possible and of significance in determining the final electrical output of the cochlear receptor is supported by anatomical studies demonstrating synaptic contacts between these fibers. Smith (1968) and Spoendlin (1966) have, for example, found synapses between efferent and afferent fibers in the region of the inner spiral bundle (ISB). In addition to the synapses made by the ISB fibers, Engstrom & Engstrom (1972) have reported that spiral tunnel bundle (STB) fibers form synaptic contacts with afferent nerve fibers in the guinea pig.

As part of a survey of neural organization in the region of the inner hair cells of the guinea pig, we have made a detailed study of the spiral tunnel bundle using both light and electron microscopy. In this investigation an effort has been made to gather additional data which may aid in assessing the significance of the STB for information processing inside the organ of Corti.

### METHODS

The present study represents an extension of our work on the inner spiral bundle (Wright, 1975). Light microscopic observations of the STB have been made on silver-stained, whole mount preparations from the 14 experimental animals used in the ISB study. These animals received lateral brain stem lesions designed to interrupt the efferent nerve

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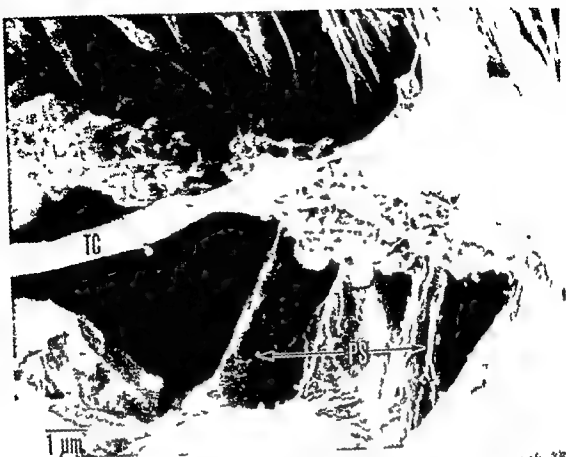


Fig. 1 Scanning electron micrograph of the spiral tunnel bundle (STB) in the second turn of a normal guinea pig cochlea. TC: upper tunnel-crossing nerve fascicle; PS: the STB; PS: stalks of inner pillar cells.

supply of the inner ear on one side. The postoperative survival times were from one to three weeks in most cases. A detailed description of the lesions, survival times and staining technique is found in our previous report. In addition to the preparations from experimental animals, the innervation was studied in whole mounts from a large collection of normal material.

Altogether 14 animals were used for electron microscopic study. Five of these had successful lateral brain stem lesions with survival times of 18 hours, 24 hours, 2 days, 5 days and 28 days. The remaining animals were untreated controls. The methods used for preparing tissue for both scanning and transmission electron microscopy are given in an earlier publication (Wright & Preston 1973).

## RESULTS

A scanning electron micrograph of the spiral tunnel bundle is shown in Fig. 1. It shows the normal position of the bundle in the second turn of the guinea pig cochlea. The STB is just inside the tunnel of Corti as stalks of the inner pillar cells near the tunnel space. In this micrograph a tunnel-crossing fascicle and numerous innervations on the individual fibers of the bundle can easily be seen.

*Light microscopic observations*  
Light micrographs of silver-stained preparations showing the tunnel bundle in the upper, middle, and basal portions of the turn of Corti can be seen in Fig. 2. With the silver stain used in this study there was a striking variation in the degree of staining.

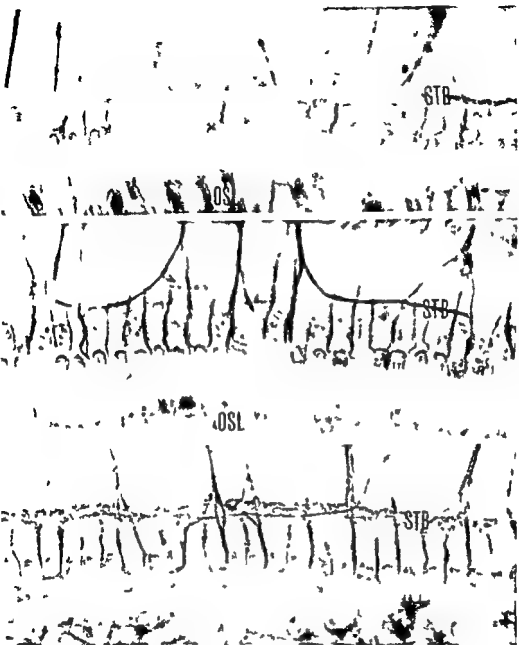


Figure 1. Micrographs of silver-stained whole mounts from normal guinea pigs showing the spiral lamina (STB) in the upper third (A), second (B), and

mid-basal (C) cochlear turns. The bars at lower left represent 10  $\mu$ m in each case. OSL, osseous spiral lamina; STB, region of inner spiral bundle.

of different components of the organ of Corti. In all turns of the organ of Corti, stained nerve fascicles were present and stood out sharply against a diffuse background of lightly stained elements. The darkly stained fibers were always

more numerous in the third and apical turns of the cochlea. In the lower turns only scattered short fascicles were heavily impregnated in the more diffusely stained substance of the bundle. The heavily stained fascicles consistently longer in the upper part



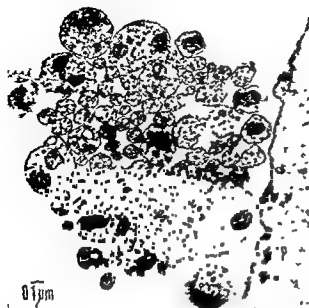


Fig 3 Transmission electron micrograph showing a cross section of the spiral tunnel bundle in the middle third turn of a normal guinea pig cochlea. In this specimen approximately 90 fibers are present in the STB. IP, inner pillar cell

cochlea than in the lower turns where the fibers usually ran for no more than 50  $\mu\text{m}$  in the STB before they left the bundle to cross the tunnel space. The long fascicles in the third and fourth turns were difficult to measure accurately since their points of entry into and exit from the bundle were often impossible to locate. However, some of these fascicles could be traced for 250 to 300  $\mu\text{m}$  before they were lost and it was apparent that longer ones were present, particularly in the apical turn. Indeed, in a preparation from the fourth turn in which most of the STB fibers had degenerated as a result of a brain stem lesion it was possible to trace a tiny fascicle (probably composed of a single fiber) over a distance of about 600  $\mu\text{m}$  toward the apex. Short fibers running for a few microns up to 40 or 50  $\mu\text{m}$  were present in all turns including the third and fourth where they were intermingled with long fascicles. Toward the lower basal part of the organ of Corti, a few darkly-stained fibers were seen in the bundle and those that were found usually had a course of less than 20 microns in the STB. It appeared that, in parallel with the inner spiral bundle (Wright, 1975),

the overall size of the tunnel bundle, reduced in the hook portion of the I. In fact, by light microscopy the seldom discernible at all in hook.

Darkly-stained fascicles were found in the tunnel bundle from the ISB toward either the base or the apex of the cochlea before leaving the STB in the tunnel. However, in the second, fourth turns fibers coursing toward the base definitely predominated. STB fibers in the basal turn appeared to run toward the base or apex in about equal numbers. For example, of 28 shorter fibers (less than 100  $\mu\text{m}$  in length) measured in the apex of normal ears, 20 ran toward the apex and 8 toward the base. In a sample of 67 fibers in the third turn, 48 were found to course toward the apex; 19 ran toward the base. Of 10 turn fibers 30 coursed in an apical direction and 12 ran toward the base. Five fibers in the basal turn 19 ran toward the apex while 16 coursed in the direction of the base.

Almost all fibers that left the spiral bundle crossed the tunnel of Corti to the cells. However, a few were found that entered the inner spiral bundle. These entered the STB from the inner space and after a short course in the STB crossed the ISB. Such fibers were rarely seen and found only in the upper turns. Also in the third and fourth turns, fibers were occasionally found to leave the STB, run longitudinally in the tunnel space, and then return to the bundle. In their longitudinal course

Table 1

Cochlear turn	Ear		
	A*	B*	C
Apical	10	40	32
Third	45	40	90
Second	95	110	60
Basal	143	155	-
Lower Basal	40	-	69

\* Ears from same animal. \* Shown in Fig 1



Longitudinal section of the spiral tunnel bundle showing a radial fascicle of fibers passing between pillar cells (IP) and entering the bundle. The

arrows indicate two large swellings on tunnel bundle fibers which contain dense-cored vesicles.

usually crossed over several radial tunnel bundles. A small swelling was often found on longitudinal fiber at the point where it crossed a radial one. Longitudinally oriented fibers were seen rather frequently in the upper part of the bundle and many of these appeared to be looping from the STB rather than crossing the bundle in an oblique course. However oblique crossings were also noted occasionally.

#### *Electron microscopic observations*

It was possible to count accurately the number of fibers present in the STB only in cross sections of a bundle examined by electron microscopy. An example of one such cross section of a particularly large tunnel bundle is shown in Figure 3. Fiber counts were made using tissue samples from five cochleas taken from four adult guinea pigs. Table I shows the number of fibers found in the various turns of the organ of Corti in these ears. It will be noted that

there was considerable variation in the size of the tunnel bundle between different animals. The STB was consistently largest in the mid-basal turn and the number of fibers in the bundle was reduced at both the apical and basal ends of the cochlea.

Electron microscopy was attempted on some of the material fixed in 10% formalin and stained with silver. As expected, this tissue was not adequately preserved for detailed electron microscopic study. However, it was obvious that some fibers, apparently scattered at random throughout the bundle, were encrusted with a particularly dense granular precipitate. These fibers apparently correspond to the darkly stained elements observed by light microscopy. With the electron microscope, no evidence was found to indicate that fibers of a particular type or in any specific position within the bundle were more likely to be heavily stained.



Fig 5 Desmosomal contact between fibers of the spiral tunnel bundle in the mid-basal turn

The large swellings frequently found on tunnel bundle fibers are filled with mitochondria and vesicles like those usually associated with synapses. We have noted in agreement with Engstrom & Engstrom (1972) that some fibers also contain large dense-cored vesicles as seen in Fig 4 which shows a longitudinal section of the tunnel bundle. Using material fixed in either glutaraldehyde or osmium tetroxide we have made a careful search by electron microscopy for synaptic contacts in the tunnel bundle. Both cross sections and longitudinal sections up to 100  $\mu\text{m}$  in length were studied in cochlear tissue from normal animals and from experimental animals with brain stem lesions. On rare occasions symmetrical membrane thickenings characteristic of desmosomes were found and an example of one such structure is shown in Fig 5. However we were unable to find any evidence of contacts which met the established morphological criteria for synapses in the spiral tunnel bundle of the guinea pig. It is also of interest to note that fibers having the morphological characteristics of cochlear afferents were never seen in or passing through the tunnel bundle.

### Brain stem lesions

The degeneration of the inner spiral bundle following brain stem lesions designed to interrupt the efferent nerve supply to one ear is described in a preceding paper (Wright 1975). It was found that the number of tunnel bundle fibers that degenerated as a result of these lesions very closely paralleled the degree of degeneration in the ISB. That is in the animals in which there was complete degeneration of the ISB there was also a total disappearance of STB fibers. Complete degeneration of both bundles occurred in those animals in which the lesions had a more medial position (Wright 1975 Fig 1 and related discussion). Some fibers remained in both the inner spiral and tunnel bundles in the animal that received the more laterally placed lesion.

In addition to the experimental material studied with the light microscope electron microscopic observations were made on cochlear tissue from 5 animals with survival times of 18 hours, 24 hours, 2 days, 5 days and 10 days. Little evidence of degeneration was seen in the tunnel bundle in the animals with 18 or 24 hour survival times although degenerated efferent terminals were noted beneath the outer hair cells. Extensive degeneration of tunnel bundle fibers was found in the animal having a 2-day survival time but some normal appearing fibers were still present. In 4 animals with survival times of 5 days and 10 days no normal STB fibers were found in a turn of the organ of Corti.

### DISCUSSION

In this investigation light microscopic observations on the organization of the tunnel bundle have been combined with electron microscopic study of its ultrastructure in an effort to obtain a more complete picture of this portion of the cochlear nerve supply.

Electron microscopy done on silver-stained preparations indicated that darkly stained fibers seen by light microscopy represent a random sample of the STB fiber population.

true, it can be concluded that the fibers upper turns tend to run predominantly in the same direction. Many of these fibers run for long distances (200  $\mu\text{m}$  or more) in the same direction.

On the other hand, tunnel bundle fibers in the basal turn of the guinea pig were found to be uniformly short and ran toward either the base or apex in equal numbers.

The fact that a larger proportion of the STB fibers is consistently stained darkly in the third and apical turns may account for Møller's (1951) observation that there was an increase in size of the tunnel bundle toward the apex in some of his silver preparations. Counts of tunnel bundle fibers, based on electron microscopy, are in reasonable agreement with those of Engstrom et al. (1966) for the guinea pig. However, we did find a considerable variation between animals in size of the tunnel bundle. The tunnel bundle was found to be largest in the middle to upper basal turn with decreases in size at both the apical and basal ends of the cochlea.

In the apical turn we were able to trace some small fascicles for distances of 250 to 300  $\mu\text{m}$ . Many of these showed no divisions or a small decrease in size within the tunnel bundle, suggesting that at least some of their constituent fibers extend the entire length of the tunnel bundle. These measurements agree with Møller's (1951) finding that some fibers are 300  $\mu\text{m}$  in the STB. It was apparent in our preparations that some fibers have longer segments in the bundle but they generally could not be accurately measured. However, one tiny fascicle was followed over 600  $\mu\text{m}$  in the efferent tunnel bundle.

Møller (1951) states that all STB fibers eventually turn out to the outer hair cells. In a recent study of zinc iodide-osmic acid-stained material (Wright & Preston 1973) we found that some tunnel bundle fibers returned to the efferent spiral bundle after running for a short distance in the STB. Such fibers have rarely been observed in our silver stained preparations. However, a few fibers were seen to

turn back into the ISB from the tunnel bundle in the third and fourth turns.

Engstrom et al. (1966) noted fine fibers running in a longitudinal fashion in the tunnel bundle of Corti in the guinea pig. Such fibers were also visible occasionally in our silver stained material. Many of these could be seen to originate from the tunnel bundle, loop out into the tunnel space, and then return to the STB. Other longitudinal fibers appeared to be crossing the tunnel to reach the outer hair cells via a long, oblique course.

Numerous enlargements or swellings are present on the fibers of the tunnel bundle. These are filled with mitochondria and vesicles which suggest the existence of synapses. In fact, Engstrom & Engstrom (1972) have reported that they found synaptic contacts in the STB of the guinea pig. We were therefore surprised that an extensive study of the tunnel bundle by electron microscopy revealed no evidence of synaptic profiles. However, symmetrical membrane thickenings such as shown in Fig. 5 were seen on a few occasions. We are inclined to agree with Spoendlin (1966), that these structures represent desmosomes rather than functional synaptic contacts. In addition, we found that all fibers of the tunnel bundle degenerate after appropriately placed lesions are made in the brain stem. This result is in agreement with the finding of Smith & Rasmussen (1963) in the chinchilla, Spoendlin (1966) in the cat, and Morrison et al. (1975) in the guinea pig. Also we were never able to locate afferent fibers within or passing through the STB. These observations indicate that the spiral tunnel bundle is composed entirely of fibers of the efferent type. There would thus appear to be little opportunity for synaptic communications between efferent and afferent nerve fibers in the STB. However, this does not rule out the possibility that efferent fibers of different types may occasionally synapse with one another within the bundle. Engstrom & Engstrom (1972) have described fibers in the ISB and STB which contain dense-cored vesicles and appear to

represent a specific group of efferent fibers. We have observed such fibers in the tunnel bundle in this study. Their exact origin and nature is, however, still unclear. In a recent investigation utilizing the squirrel monkey, Nakai & Igarashi (1974) found the tunnel bundle to be composed of fibers from both the crossed and uncrossed portions of the efferent nerve supply. It might be noted that preliminary experiments in our laboratory indicate that this is also the case in the guinea pig.

It is of interest to recall that the STB is not a constant feature of cochlear innervation among the mammals. The bundle is present, for example, in some species of rodents, such as the guinea pig and chinchilla, and absent in others such as the rat (Smith & Haglan, 1973). It may be that in those animals in which the tunnel bundle is present it simply represents an outlying portion of the inner spiral bundle made up of efferent fibers destined to innervate the outer hair cells. Any significant synaptic interaction between afferent and efferent neural elements is probably confined to an area closer to the habenula perforata within the inner spiral bundle proper.

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### ZUSAMMENFASSUNG

Die Organisation der Nervenfasern im spiralen Tunnelbündel (STB) des Cortischen Organs von Meeresschweinchen wurde licht- und elektronenmikroskopisch untersucht. In der basalen Windung verliefen die STB-Fasern in etwa gleicher Anzahl wie zur Apex, während sie in den oberen Windungen vorwiegend apikal verliefen. Lange Spiralfasern mit Myelin oder Langer waren häufig in der 3. und 4. Windung, aber selten in den unteren Windungen. Zwischen den Fasern des Tunnel-

bündels konnten keine synaptischen Kontakte gefunden werden und ebenso wurden keine afferenten Nervenfasern beobachtet.

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## INNER EAR DAMAGE AND HEARING LOSS AFTER EXPOSURE TO TONES OF HIGH INTENSITY

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Experimental animals (cats) were exposed to 125 1000 2000 and 4000 Hz at sound pressure in the range 120 to 157.5 dB and for durations of 1000 2000 4000 Hz) or four hours (125 Hz) before audiograms were obtained for each animal before and after exposure. Post-exposure tests were conducted until complete recovery of hearing had occurred or a stable permanent threshold shift had been observed. Cochleas of animals were examined by contrast microscopy. Condition of all hair cells was determined. Extent of inner-ear damage and range of frequency for which hearing loss occurred increased as sound pressure level was decreased in frequency. For example, exposure to 4000 Hz produced damage in a restricted range of the cochlea and hearing loss for a relatively narrow range of frequencies. Exposure to 125 Hz produced widespread inner ear damage and hearing loss throughout the frequency range 125 to 6000 Hz.

In the early days of experimental studies of hearing, investigators have used tones or noise of high intensity to stimulate the ear of an experimental animal and thus, produce damage to structures and change in function of the inner ear. In a review of the literature on the problem of stimulation deafness, Kemp (1967) cited 44 published reports of clinical and

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experimental investigations. The earliest clinical study mentioned that went beyond casual observations was published in 1890 (Habermann), the earliest experimental study, in 1907 (Wittmaack).

With the development of new techniques of examining inner ear pathology and of measuring inner ear function, important new information has come repeatedly through the use of this old procedure which allows physical characteristics of the exposure stimulus to be correlated with inner ear pathology, stimulus characteristics, with changes in inner ear function, and inner ear pathology, with changes in inner ear function.

Major advances in methods of detecting and mapping inner ear damage have included (in more or less chronological order) 1) improved techniques of sectioning and staining of mammalian cochleas, 2) graphic reconstruction of cochleas from serial sections, 3) surface preparation of organ of Corti and use of phase-contrast microscopy to examine and map all hair cells, 4) examination of selected regions of organ of Corti by transmission electron microscopy, 5) examination of cochlear structures by scanning electron microscopy.

The important methodological advances in studying inner ear function have been 1) recording electrical responses produced by inner ear elements, 2) use of behavioral methods

obtain accurate measures of auditory discrimination (absolute and differential thresholds)

In the majority of published reports, inner ear damage has been assessed and related to frequency, intensity, duration and complexity of the sounds used to produce damage, or change in inner ear function as measured by electrophysiological or behavioral techniques has been related to physical aspects of the exposure stimulus. There have been a relatively small number of studies in which inner ear damage and inner ear function have been examined in the same animal after controlled exposure to sound stimuli. In the earliest of these experiments, inner ear pathology was assessed by examination of selected serial sections through the cochlea, graphic reconstructions were made according to the method of Guild.

Bredberg & Hunter Duvar (in press) have recently published a summary of the results of animal experiments and human studies in which changes in hearing have been related to inner ear damage that was produced by exposure to high intensity sounds or by other means, such as surgery or administration of ototoxic drugs.

Through the use of the surface preparation of the cochlea and examination by phase contrast microscopy a more precise and complete picture of damage to the organ of Corti can be obtained. In a preliminary report, Dolan et al (1970) described the results of an experiment in which the hearing of cats was tested before and after exposure to pure tones at high sound pressure levels. After sacrifice, surface preparations of the cochleas were made and all hair cells and adjacent structures were examined by phase contrast microscopy. Details of this study including results not available at the time of the preliminary report are given below.

## METHODS AND PROCEDURE

The general scheme of the investigation included pre-exposure audiometric testing con-

trolled exposure to a potentially destructive stimulus, post exposure audiometric tests and post-mortem examination of the inner ear.

Prior to initial training of the cats, one ear of each animal was destroyed by surgery. Additionally, the pinna of the experimental animal was removed and the surrounding area reconstructed. Removal of the pinna was advantageous in that it decreased the variation in intensity of acoustic stimuli at the ear due to positional changes of the pinna during behavioral testing. The absence of a pinna allowed close examination of the ear prior to and following exposure and monitoring of acoustic wave-forms during exposure, and easy accessibility for cleaning the external canal.

## Exposure

For exposure to a high intensity sound (125 Hz) each experimental animal was anesthetized with diaibutal and positioned in a stereotaxic instrument. Its head was held only by the ear bar.

The sound was generated by a sine wave oscillator. The output of the oscillator was passed through an Altec 1569 A amplifier and an Altec 802 D speaker. The output of the speaker passed through an adapter consisting of telescoping brass tubes (this allowed tuning by varying the length of the tube) finally into a speculum that was inserted into the pinnectomized ear. The sound intensity was monitored through a 1 mm probe placed just beyond the orifice of the speculum. The probe was connected to a 1/2 inch 1

power supply) and fed into a General sound vibration analyzer. Sound and vibration data were calibrated with a B&K pistonphone and probe calibration couple.

For the data presented in the Results section, frequencies of 125 Hz, 1000 Hz, 2000 Hz, and 4000 Hz were examined. At

ency, the effects of sound pressure levels also investigated

#### *Behavioral testing*

Audiograms were obtained by an avoidance conditioning procedure. In order to avoid the experimental animal was trained to move from one side to the other of a double cage when a series of tone pulses was presented. Each tone pulse had a duration of one second and rise and fall times of 300 msec.

The interval between pulses was one second. On any given trial, the tone pulses were presented for a period of ten seconds followed by shock given through the bars that divided the floor, sides, and top of the double cage.

The tone pulses were generated by a General Radio 1310-A oscillator, amplified by a Teknosh MC-250 amplifier, gated without retarding to phase by an electronic switch (Olsen and Ludwig, 1965), and presented via an Lansing 802 D speaker. The loudspeaker and double grill cage were located in a sound-deadened, double-walled room. Sound conditioning and control instruments were outside of the room. The experimenter sat at a control panel and could observe the experimental animal through a one-way window.

After an animal had learned to make avoidance responses to tones well above threshold, the minimum sound pressure level (SPL) at which the animal would respond was determined at each of the following frequencies: 250, 500, 1000, 2000, 4000, 8000, and 16000 Hz. The general procedure for determining a "threshold" for a given frequency involved the attenuation of the tone by 15 dB for each correct response. When a level was reached at which no response was made, the level was increased by 15 dB and then attenuated in 5 dB steps until another failure occurred. This procedure was repeated until the animal reversed his behavior from "responding" to "not responding" in the same 5 dB step twice in succession. Following determination of a "threshold" in this manner,

the tone was increased by 50 to 80 dB and the entire procedure repeated. Two "thresholds" were obtained at a single frequency during each test session. Pre-exposure testing was continued until stable audiograms had been obtained, that is, until the range of threshold values at each frequency had decreased to 10 dB or less for several successive tests.

A similar procedure was followed after exposure to a high intensity tone. Audiograms were measured until recovery to normal pre-exposure levels occurred or until the amount of measured threshold shift remained constant for a period of several weeks. The time from exposure until sacrifice varied from two to four months for the animals included in the present report.

#### *Post-mortem anatomical methods*

The preparation of the cochleas for post-mortem examination was essentially similar to that described in detail by Engström et al. (1966), except that the entire organ of Corti was displayed on slides as surface specimens. All hair cells were counted and the results recorded on punch cards for computer analysis. The resultant cochleograms gave a picture of the remaining or intact hair cells.

## RESULTS

The behavioral data showing amount of hearing loss (PTS, permanent threshold shift) and the anatomical data showing the percent of hair cells missing as a result of the exposure to a tone of high intensity are summarized below for 16 animals. The amount of hearing loss suffered by an animal at a particular frequency is the difference in the median value of the final six measures obtained prior to the exposure and the final six measures prior to post-mortem examination.

#### *Exposure to 4000 Hz*

The behavioral and anatomical results for three animals exposed to a 4000 Hz tone are



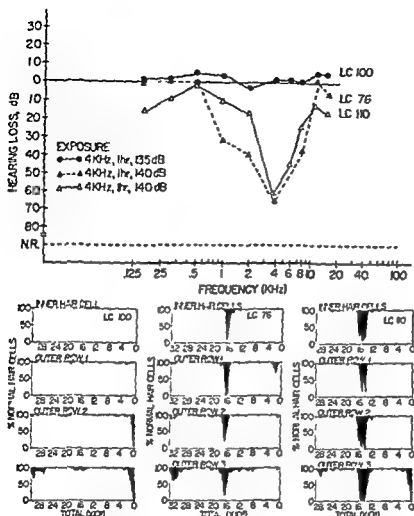


Fig. 1. Pure tone thresholds (upper half of figure) and plots of inner ear damage (lower half of figure) for animals exposed to 4000 Hz tone. For each animal, the 0-dB line is its preoperative hearing level. Hearing loss or permanent threshold shift (PTS) is plotted as a deviation from the 0 line. In the plots of inner ear damage, the base line (labelled 0, 4, 8, 12 etc.) indicates the number of hair cells as counted starting at the base of the cochlea. For example, 4 means that at that point four hundred hair

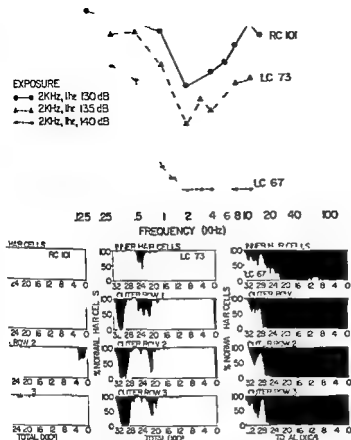
cells had been counted. The number of destroyed or aged hair cells is shown by the black shaded area. For example, in the plot for cat LC 110, all of the hair cells in the outer row 3 were damaged in the region occupied by cells 1400 to about 1600 (upper basal turn). The damage was less in the other two rows of outer hair cells and in the single row of inner hair cells. NR = No response at maximum level available.

shown in Fig. 1. The top half of Fig. 1 shows the amount of hearing loss (relative to pre-exposure audiogram) as a function of frequency. The boxes in the lower half of Fig. 1 show the percentage of normal hair cells remaining after the exposure as a function of the total cumulative number of hair cells measured from the basal end of the cochlea. Region and extent of absent or damaged hair cells are indicated by

blackened areas. All three animals received exposure of one hour duration. Cat LC 100 was exposed to a 135 dB SPL tone, cats LC 76 and LC 110, to a 140 dB SPL tone.

As indicated in Fig. 1, cat LC 100 suffered no hearing loss as a result of the exposure. Cats LC 76 and LC 110 had hearing losses in the frequency range 500 to 12000 Hz, the maximum loss occurring at the exposure frequency. At 4000 Hz, there was a permanent hearing loss of approximately 60 dB.

† All sound pressure levels are expressed in dB re 0.00002 pascal.



■ tone thresholds and inner ear damage of animals exposed to 2000 Hz (Fig. 1 for explanation)

It also shows that cat LC 100 sustained only no hair cell destruction as a function of the exposure. Only slight damage was found along the outer (3rd) row of outer hair cells. Cats LC 76 and LC 110 had but narrow lesions in all three rows of hair cells and in the single row of inner hair cells lesions occurred in the upper basal turn midway along the length of the cochlea.

#### Results of exposure to a 2000 Hz tone

Results of exposure to a 2000 Hz tone that are shown in Fig. 2.

RC 101 was exposed to a 130 dB SPL tone and sustained a hearing loss in the range of 2000 to 12000 Hz with a maximum loss of

about 38 dB occurring at the frequency of the exposure tone. No hearing loss was found for either the very low or very high frequencies. Cat LC 73 exposed to a 135 dB SPL tone had greater hearing loss than RC 101 for all frequencies from 500 Hz to 12000 Hz. Again the maximum loss about 58 dB occurred at the frequency of the exposure tone. Cat LC 67 was exposed to a 140 dB SPL tone. It had complete loss of hearing for frequencies from 2000 to 12000 Hz, severe loss at 1000 Hz and smaller losses at 500 and 250 Hz.

The anatomical results of the exposures to a 2 kHz tone are given in the lower half of Fig. 2. Cat RC 101 which had a 38 dB hearing loss at 2000 Hz had only minor hair cell damage. With the exception of a very narrow lesion in

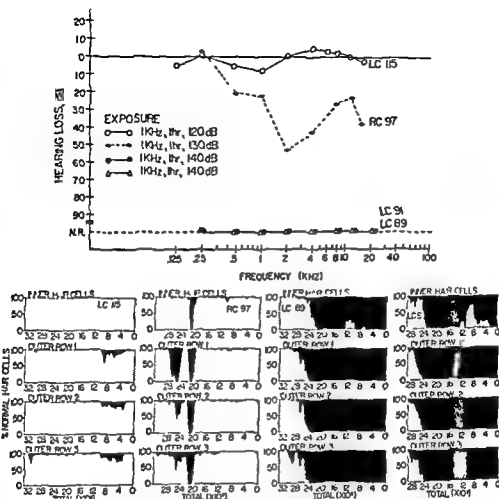


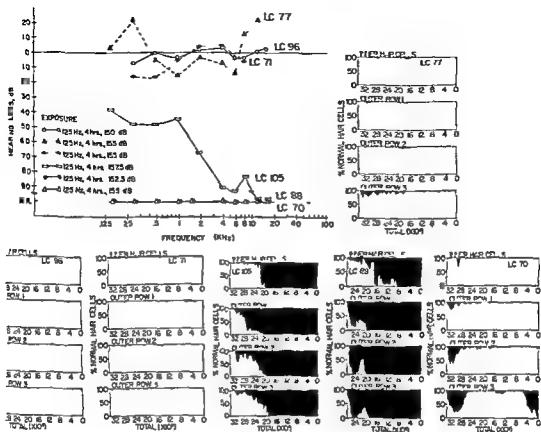
Fig. 3 Pure tone thresholds and inner ear damage for animals exposed to 1000 Hz (Fig. 1 for explanation)

which approximately 40% of the OHCs in the first row were missing, cat RC 101 appeared to have a nearly normal cochlea. Cat LC 73, which had a 58 dB hearing loss at 2000 Hz, had severe destruction of both inner and outer hair cells, the greatest damage to the outer hair cells occurred in two regions toward the apical end of the cochlea. One region of severe damage was in the approximate location of maximal activity caused by a 2000 Hz tone, the second region was at the apical tip of the cochlea and is apparently not reflected in the audiometric data. It should be noted that the lesion that occurred more distant from the apical end is much like the lesions found in cats LC 76 and LC 110 as a result of exposures to a 4 kHz tone, the lesion is confined to a very limited region of the basilar membrane.

The anatomical results of cat LC 67 suffered the greatest PTS as a result of 2000 Hz exposure, are also shown in Fig. 3. As expected from the audiometric data, LC 67 had complete destruction of IHC of all three rows of OHCs in all but the tip of the cochlea.

#### Exposure to 1000 Hz

The audiometric results for four animals exposed to a 1 kHz tone for one hour are shown in Fig. 3. Exposure levels were 120, 130, and 140 dB. Cat LC 115 was exposed to a 120 dB SPL tone and had no PTS. Cat RC 97 was exposed to a 130 dB SPL tone and had a moderate PTS at all frequencies above 250 Hz, the maximum loss occurring at 2000 Hz. Cats LC 91 and LC 89 were both exposed



Pure tone thresholds and inner ear damage for cats exposed to 125 Hz (Fig. 1 for explanation)

SPL tone and, after exposure, failed to respond to any signals between 250 and 20000 Hz. Audiometric tests were made at 125

Hz. Anatomical results for the four animals exposed to 1000 Hz are shown in Fig. 3. Cat LC 5 had essentially no damage as a result of exposure. Cat LC 97 had a double-headed lesion of the outer hair cells and a narrow region of damage to inner hair cells. Cats LC 91 and LC 89 both suffered total loss of OHCs and IHCs throughout all of the apical end of the cochlea.

#### Exposure to 125 Hz

Audiometric results for six animals exposed to 125 Hz for four hours are shown in Fig. 1. Cat LC 96 was exposed to a 150 dB SPL tone and suffered no PTS. Cats LC 71 and LC

77 were exposed to a 155 dB SPL tone and, like LC 96, had normal post exposure audiograms. Cat LC 105, exposed to a 157.5 dB SPL tone, had severe PTS for frequencies from 125 to 16000 Hz, the amount of PTS being greater at the high frequencies. Cats LC 88 and LC 70, which were exposed to intensities of 152.5 dB SPL and 155 dB SPL, respectively, failed to respond, after exposure, to tones throughout the range 125 to 16000 Hz.

The anatomical results for the animals exposed to 125 Hz are also shown in Fig. 4. Cats LC 77, LC 96, and LC 71 suffered little or no damage from the sound exposure. Cat LC 105 had complete loss of hair cells in the basal half of the cochlea and partial loss of cells in the upper turns. Cat LC 88 had almost total loss of OHCs and extensive damage to IHCs. Cat LC 70 had a nearly normal cochlea, limited damage

age occurred to outer hair cells at the apical end of the cochlea and to a small percentage of inner hair cells near the apex. There was also loss of OHCs in row 3 near the round window.

## DISCUSSION

### *Locus of damage—frequency of exposure tone*

The relationship between frequency of exposure and locus of inner ear damage is most evident in those animals in which the exposure caused only a narrow region of damage. As a result of exposure at 4 kHz, for example, animals LC 76 and LC 110 sustained narrow but severe lesions of both OHCs and IHCs in the upper basal turn of the cochlea. In cases in which the exposure frequency was lowered to either 2000 Hz (LC 73) or 1000 Hz (RC 97) however, the region of hair-cell loss occurred at locations further from the basal end of the cochlea (approximately in the middle turn). In animals that suffered severe hair cell destruction after exposure to either 2000 Hz or 1000 Hz (LC 67, LC 89, LC 91), all hair cells were destroyed from the upper middle or lower apical turn to the apical end of the cochlea.

Exposure to 125 Hz, in all but one case produced either no damage (LC 77, LC 96, LC 81) or widespread destruction of hair cells (LC 103, LC 88) throughout the cochlea extending from the basal turn to the apical end.

### *Inner ear damage—exposure level*

For frequencies of 4000, 2000, and 1000 Hz, the relationship between the amount of inner ear damage and the sound pressure level of the exposure tone is clearly seen: the extent of damage was, without exception, greater in those animals that received the highest exposure levels.

There was greater variability of results for exposure to 125 Hz, nevertheless the two animals with greatest inner ear damage were exposed to sound pressure levels as high or higher than the exposure levels of animals with less inner ear damage.

More interesting, perhaps, was the amount of change in the exposure required to alter the resultant cochlear damage from minimal to severe. At all exposure frequencies investigated, for example, a small increase in the intensity of the exposure caused the difference between moderate and severe destruction in the cochlea. At 125 Hz, a change of less than 5 dB in exposure intensity sometimes meant the difference between a normal and a damaged cochlea.

### *Hearing loss—exposure level*

For exposures to 1000, 2000, and 4000 Hz, hearing loss increased with increase in exposure level. Again, there was great variability in results for animals exposed to

### *Hearing loss—region and extent of inner ear damage*

For animals exposed at 1000, 2000, and 4000 Hz, the frequency at which maximal hearing loss occurred corresponded well with the locus of hair-cell damage. Often, however, a range of frequencies for which hearing loss occurred did not correspond well with the width of the cochlear lesion (particularly narrow cochlear lesions). Animals with maximal hearing loss at 4000 Hz (LC 76 and LC 110), for example, had damage to both OHCs and IHCs in the upper basal turn of the cochlea. Animals with maximal hearing loss at 1 kHz (RC 101 and LC 73) had hair cells destroyed in the middle turn of the cochlea (although LC 73 had a second lesion at the apex). The animal with maximal hearing loss at 1 kHz (RC 97) also had lesions in the middle and apical turns. In each of these cases, the range of frequencies at which hearing loss occurred was greater than would have been expected based on the width of the cochlear lesion or lesions. There are several possible explanations of this discrepancy. The most likely is that the histological data presented are based primarily on a determination of

“presence” or “absence” of each using phase-contrast microscopy. No attempt was made to ascertain the number of a hair cell. More subtle alterations of a hair cell than its elimination or severe loss would not be seen although other methods such as electron microscopy might have revealed damage sufficient to render the hair cell non-functional. This explanation would account for such discrepancies as reported by RC 101 which had considerable hearing loss but only minimal hair cell damage. Losses sustained at the apical end of the cochlea of LC 73 and RC 97 may have been caused by interruption of the blood supply in the apical region, a disorder that might be related to the sound exposure (see Bernstein et al., 1967).

In cases in which the exposure caused total destruction of OHCs and IHCs (LC 88, LC 91, LC 89, LC 67), there was corresponding severe loss of hearing at low frequencies or complete loss at higher frequencies and severe loss extending into the middle frequency-range. In one case, LC 70, the exposure hearing loss cannot be explained in terms of inner-ear damage.

#### *loss—destruction of OHCs?*

As obtained in this experiment do not have any definitive answer to the question: “Do audiograms reflect the condition of the inner ear cells, outer hair cells or both?” Evidence from several animals is relevant to this question. Cat LC 88 had almost complete destruction of OHCs but a large number of IHCs in the apical turn were still present, post-exposure testing indicated complete loss of hearing. LC 91 also had complete loss of hearing although some IHCs remained in the apical and basal turns. RC 101 had post exposure hearing loss for frequencies from 2000 to 4000 Hz although IHCs remained throughout the cochlea.

In the above cases (LC 88, LC 91, and RC

101) might be taken as evidence that the audiogram does not necessarily reflect the condition of the IHCs.

In contrast to the above cases, LC 73 had normal hearing for low frequencies despite the nearly total destruction of OHCs in the apical turn, IHCs were intact in the apical turn and throughout the cochlea except for a narrow region in the middle turn. In this case, it might be argued that the audiogram does reflect the condition of the IHCs.

Finally, in considering the results of the present study and of many others that have related hearing loss to inner-ear damage, the limitations of methods used in assessing inner-ear damage must be kept in mind. No single histological technique provides a complete assessment of intracochlear damage or change resulting from an exposure. It is necessary to accurately assess damage to hair cells, intracochlear changes such as mechanical destruction of nerve fibers, disruption of the synaptic region between sensory cell and nerve fiber, interruption of blood supply, and damage to supporting structures.

There are also limitations in the methods used to measure changes in hearing in experimental animals. Occasional anomalous results may be expected. In an animal with a severe hearing loss, it may be difficult to obtain threshold measurements. For a normal animal or one with moderate hearing loss, tests of absolute threshold may be started by presenting a tonal signal well above threshold and decreasing it until the animal no longer makes a behavioral response. For animals with severe hearing loss, it may not be possible to use a tonal stimulus that is much above threshold. Therefore great care must be taken not to produce “neurotic” behavior by creating highly stressful conditions for an animal—particularly one that has been exposed to loud sound or has otherwise been treated so as to produce a severe hearing deficit. The presence of tinnitus may also disrupt the animal's performance in response to weak tonal signals.

## ACKNOWLEDGEMENTS

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## ZUSAMMENFASSUNG

Katzen wurden reinen Tönen von 125, 1000, 2000 und 4000 Hz mit einer Schallintensität im Bereich von 120 bis 157,5 dB SPL, während einem Zeitraum von 1 Std (1000, 2000 4000 Hz) bzw. 4 Std (125 Hz) ausgesetzt. Vor und nach jedem Versuch wurde von jedem Tier ein Tonaudiogramm erstellt. Nach Schallexposition wurden die Hörtests so lange wiederholt, bis feststand, dass die normale Hörfähigkeit wieder erlangt war oder ein bleibender Hörverlust festgestellt werden konnte. Die Cochlea der Tiere wurde im Phasenkontrastmikroskop untersucht. Der Zustand aller Haarzellen wurde untersucht und ausgewertet. Das Ausmass des Innenohrschadens und die Frequenzbreite, in der ein Hörverlust auftrat, wurden grösser, wenn die Tiere Tönen niedriger Frequenz ausgesetzt wurden. Beispielsweise wurde beobachtet, dass eine Schallexposition von 4000 Hz Haarzellschaden in einem kleinen Abschnitt der Cochlea hervorrief und einen Hörverlust in einem eng begrenzten Frequenzbereich verursachte. Nach Exposition von 125 Hz wurde ein aus-

gedehnter Innenohrschaden gefunden mit Hör Frequenzbereich von 125-6000 Hz

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## AUDIOLOGICAL FINDINGS IN 125 CASES OF ACOUSTIC NEUROMAS

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The audiological findings in 125 patients with confirmed acoustic neuromas are presented. The classification by Pulec et al (1971) was used. 105 large tumours, small tumours were not represented. A clear connection between hearing loss and tumour size was noted. There was no correlation between duration of tumour size. An attempted evaluation of the audiological tests (ABLB and Metz recruitment, discrimination scores, tone decay, reflex decay tracings) that were applied to the patients has been made. Attention is called to some audiological findings which in our knowledge have not been described previously. No patient in the entire material had hearing 73 had anacusis and 52 hearing varying degree. In the presence of a normal ear the evaluation of audiological tests at hearing loss poorer than 80 dB is rather questionable. It was therefore concentrated on 32 patients with hearing loss of 80 dB or less. The pathophysiology for the typical hearing loss in patients with acoustic neuroma is a reduction in the number of axons in the acoustic nerve and it was to be expected that abnormal findings would be present especially in tests that exert the greatest demand on the transmission capacity of the nerve. In other words application of intense and/or prolonged sounds. The test is just such a procedure and it is not surprising that it shows the highest degree of validity between the tests. No single test suffices to distinguish between retrocochlear disease and it is necessary to apply a battery of tests. Any unexpected variability in the results of ordinary routine test results has gradually become the main indication to pursue the diagnosis with more elaborate procedures and it has been a great help to apply the Metz test as a part of our routine examination.

A close connection between the size of the acoustic neuroma and the complications, especially mortality at operation. Early

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diagnosis is vital for the patient. Though much improved over the past 25 years, our efforts to detect these tumours at an early stage have by no means paralleled the advances made in surgical treatment of acoustic neuromas. This is very regrettable since in most cases it is now possible to remove small tumours with very low mortality and little or no damage to the facial nerve.

This paper presents the audiological findings in 125 patients with surgically confirmed acoustic neuromas. We have attempted an evaluation of the usefulness of the various audiological tests that were applied to the patients. Attention is called to some of the audiological findings, which to our knowledge have not been described previously. We also present a case history, with an—unfortunately—not infrequent course.

### METHODS

The classical ABLB-test continues to be a very important tool in the differential diagnosis of perceptive disorders and it was applied systematically to all patients where the hearing loss did not exceed 80 dB PTA.

The Metz recruitment test, based on the middle-ear muscle reflex thresholds, was introduced in the world literature from this department in 1952 (Metz, 1952). Over the years it has been applied with several types of



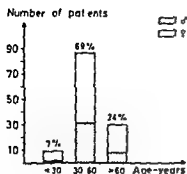


Fig 1 Age distribution of 125 patients with acoustic neuromas

equipment to all patients unless middle-ear pathology rendered it unsuitable

Speech audiometry was not readily available in our department before 1962 and was omitted in some patients early in the series. Other tests were used more sporadically, mostly as a part of specific time limited scientific projects, such as the Békésy audiometry procedures and the SISI-test, as described by Jerger (Jerger et al., 1959), various types of tone-decay testing (Carhart, 1957; Owens, 1964; Sørensen, 1962), and now in recent years the reflex-decay test (Anderson et al., 1969).

## MATERIAL

The material consists of 125 consecutive patients with acoustic neuromas, operated on in the Department of Neurosurgery, University Hospital (Rigshospitalet), in the period 1957-72. Exact measurements of the tumour size were difficult to obtain, and there are

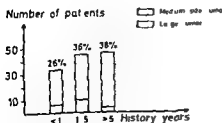


Fig 2 Length of history in relation to tumour size in 125 patients with acoustic neuromas

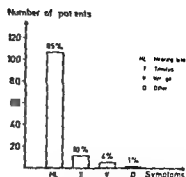


Fig 3 First subjective symptoms in 12 acoustic neuromas

often discrepancies in the way o neurosurgeons interpret the findings.

In the following, we have adopted classification described by Pulec et al. (1969). *Small* tumours (intracanalicular) are confined to the internal auditory canal. *Medium* size tumours extend into the cerebello-pontine angle without fifth nerve involvement. *Large* tumours should be no symptoms from the cerebellum. In *large* tumours, the fifth nerve is involved, causing brain stem symptoms. The intracanalicular tumours may eventually be increased to these criteria there were 20 small and 105 large tumours in our material. Small tumours were not represented.

The age distribution is seen in Fig 1.

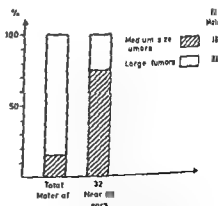


Fig 4 Distribution of tumour size in total material and in 37 patients with history of <1 year

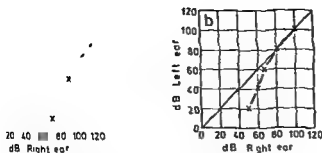
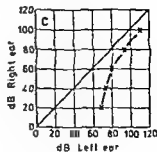


Figure 4b ABLB recruitment test results in 3 patients with Metz recruitment test



ty of patients fell within the 30–60 year age group, tumours were rare below 30 years. Women outnumbered men by two to

■ 2 the duration of symptoms is related to tumour size. Only 26% of patients were operated within the first year and 38% had a duration exceeding 5 years. There is a tendency towards a shorter history for the medium sized tumours, but the variability is large and there is no certain connection between length of history and tumour size.

3 categories of the first symptoms reported by the patients according to type. In 70% hearing loss was the initial symptom, 20% tinnitus and 4% vertigo. The remaining one patient reported an initial reduction of facial sensitivity. There were 7 bilateral tumours, and while neuromas as a whole are rare below the age of 30 (Fig. 1) in our series 25% we found that 4 out of 7 patients with small neuromas were below that age. All 4 young patients had Recklinghausen's disease while the remaining 3 had no signs of disease. A total of 6 patients suffered from incontinence of the neuroma (5%).

One patient in the entire material had normal

hearing. Of the 125 patients, 73 had anacusis, and 52 hearing loss of varying degree. This paper is mainly concerned with the various audiological tests applied and we decided for practical reasons to concentrate our attention on 32 patients with a hearing loss of 80 dB or less.

In the presence of a normal contralateral ear, we consider the evaluation of audiological tests at PTA thresholds poorer than 80 dB to be rather questionable.

While there is a great preponderance (84%) of large tumours in the material as a whole, the medium-sized tumours dominate (75%) in the selected material (Fig. 4). Thus there is a clear connection between the degree of hearing loss and tumour size. This is in contrast to the lack of correlation between duration of history and tumour size.

The 32 patients had a mean PTA air conduction threshold of 54 dB. There was good concordance between the SRT (Speech Reception threshold) and the PTA.

Table II ABLB and Metz recruitment tests in 30 patients with surgically confirmed acoustic tumours

ABLB		Metz	
+recruitment	-recruitment	+recruitment	-recruitment
4	23	4	23
3		1	
23%	77%	13%	87%

Table I Speech discrimination scores in 24 patients with acoustic tumours

	0–20%	20–40%	40–60%	60–80%	80–100%
Number	10	2	3	5	3
Mean	10 dB	42 dB	58 dB	50 dB	45 dB

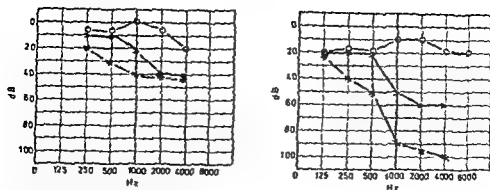


Fig 6 Pure tone audiograms obtained with an interval of one month illustrating the threshold shift when masking the non tumour ear

Table I shows speech discrimination scores (SDS) in 24 patients. There is no relation between SDS and PTA. Only 8 patients (33%) had speech discrimination scores better than 60%.

In Table II the ABLB and Metz recruitment tests are compared in 30 patients, 7 (23%) of the patients had positive recruitment with the ABLB test. Of these 7 patients 4 also exhibited recruitment by the Metz test. In the remaining 3 patients there was a distinct discrepancy between the findings by the two methods, in favour of the Metz method. The results of the ABLB tests in these 3 patients are illustrated in Fig 5a, b, c.

**Patient a** A 53-year-old woman with a progressive hearing loss in the right ear for 4 years. She had been dizzy for about 1 year prior to the first examination. The ABLB test shows positive recruitment of the delayed type. At surgery she had a large acoustic neuroma with impression in the brain stem.

**Patient b** A 16-year-old man with history of hearing loss and tinnitus in ear. There was no vertigo. The AS showed complete recruitment. A large was removed from the cerebello-ponle, it had caused some impression on stem.

**Patient c** A 26-year-old woman history of hearing loss for 1½ years. a interference with the facial sensitivity side. She showed a classical recruitment phenomenon at ABLB. There was a size tumour but no impression on stem.

#### Adaptation tests

Fifteen patients were examined for presence of tone decay. Eleven of these (73%) a type of decay typical for retrocochlear ease while the outcome was negative (27%). The results from Bekesy tracings patients are seen in Table III. 30%.

Table III Classification of Bekesy tracings in 10 patients with acoustic tumours

Type	No	%
I	1	10
II	2	20
III	3	30
IV	4	40

Table IV Patients with positive results in audiometric tests consistent with site of

Test	No tested	Percent (%)
Speech discrimination	24	58
ABLB	10	77
Metz	10	87
Tone decay	15	73
Reflex decay	5	60
Bekesy audiometry	10	70



PTA threshold shifts in relation to hearing loss in ear with no masking of non tumour ear

or II tracings, while the remaining 70% of type III and IV

patients could be tested for stapedius decay (Anderson et al, 1969), 3 had a decay, but in 2 patients the reflex at 500 remained stable for a period of 10 sec

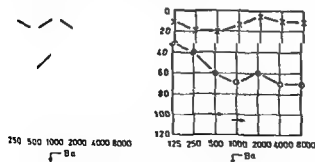
IV shows the results of the six audiometric tests that were applied in this series percentages of correct results, being consistent with presence of a retrocochlear type lesion are indicated. It is obvious that the recruitment test shows the greatest utility in the diagnosis of the retrocochlear hearing loss

15 of the 32 patients we observed a considerable threshold shift during pure tone audiometry when masking was applied to the lateral ear. This is illustrated in Fig 6

where two pure tone audiograms obtained in one patient with an interval of one month are shown. This case serves to illustrate the great threshold shift that may occur with adequate masking of the contralateral ear (overhearing to the unmasked ear is out of the question), and also the big difference in audiograms even within such a short interval. This is a characteristic feature which we observed quite frequently in pure tone audiometric examinations of patients with acoustic neuromas.

Fig 7 shows the number of PTA threshold shifts that occur with adequate masking of the non tumour ear, as related to the unmasked PTA thresholds in 15 patients. There is a correlation between shift of threshold and the level of the unmasked PTA, the poorer the unmasked PTA threshold, the greater the threshold shift with contralateral masking.

In 2 patients we noticed a symptom which to our knowledge has not been reported previously (Fig 8). The contralateral left ear was normal in both patients, but nevertheless it was impossible even with the use of maximum tonal stimuli at this ear to demonstrate the presence of a middle-ear reflex on the tumour side. On stimulation of the tumour ear a muscle reflex was found in the normal ear, but as might have been expected, only with stimulus intensities above normal.



	Left ear	Right ear
SRT	10 dB	40 dB
SDS	92%	8%

	Left ear	Right ear
SRT	15 dB	60 dB
SDS	95%	36%

Fig 8 Audiograms from 2 patients with acoustic neuromas and normal non tumour ear. No middle ear reflex could be demonstrated in tumour ear even with maximum tonal stimuli in the non tumour ear.

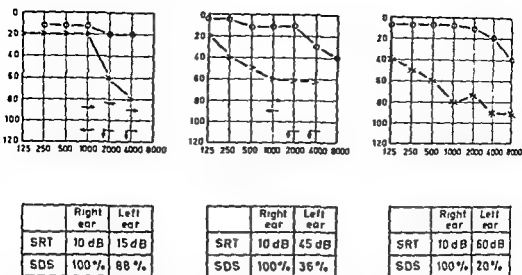


Fig 9 Audiograms in patient with acoustic neuroma (case history)

In one patient this absence of muscle reflex on the tumour side, together with the need to use abnormally high stimulus intensities on this very side before a reflex could be recorded in the normal ear, initially made us suspect that she suffered from unilateral otosclerosis accompanied by a considerable perceptive component.

Finally we want to report a case history with a course that unfortunately may occur not infrequently. The patient was a 54-year old woman. When we first saw her, she had noticed a slight hearing loss and tinnitus in the left ear for 4 months. The audiogram is seen in Fig 9. The differential-caloric test, taste and naso lacrimal-reflex examination, corneal and facial sensitivity, were all normal. There was pronounced recruitment at ABLB test and no tone-decay. Discrimination scores were high. The only abnormal finding was absence of recruitment as measured by the Metz test. The case was regarded as one of Meniere's disease and it was decided to re-examine the patient after one month. Because of lack of symptoms, however, she failed to report until after the lapse of 6 months. She had then experienced a rather sudden onset of severe vertigo. The audiogram, shown in Fig 9 demonstrated

a deterioration of hearing. Caloric reaction had disappeared and the naso lacrimal reflex was also impaired. Taste examinations were still normal. There was recruitment at ABLB and the Metz test now represents a borderline case. Other findings were pronounced tone-decay and a Békésy type IV tracing. Lophodylate cisternography was thought to demonstrate an intracanalicular tumour. This was removed by the suboccipital approach and was found to be of medium size extending 1 cm into the cerebello pontine angle. During the operation the facial nerve became injured, resulting in total paralysis, but otherwise the course was uneventful.

## DISCUSSION

Nowadays the reliability with which so-called recruitment tests are able to differentiate between cochlear and retrocochlear lesions is being re-evaluated. We like to think that this doubt as to the diagnostic validity arises because we now see the patients at an earlier stage of the disease. This seems not to be true in the present series, however, where the tumours were as large as ever, when seen at surgery. Still we find the same ambiguity in the

sults as reported by others. A common feature is the considerable test-retest variability in individual patients.

The crux of the matter appears to be the fact that it is usually quite apparent, when an ear is being tested, but far more difficult to prove that a lesion is not present. Patients with retrocochlear lesions become grouped with those who do not cooperate to a satisfactory degree. Often it is the examiner's first impression that he is faced with malingering. Considering the individual tests it is apparent that it is difficult to distinguish between those that are tested at the threshold of hearing, and those that are tested in threshold procedures.

Even tone audiometry will always be the first step. Even at this stage the true diagnosis may be suspect because of considerable uncertainty about the result. The patient will often report that it requires considerable effort to concentrate on the detection of tones at threshold intensities and the introduction of moderate masking to the contralateral ear is a more than normal degree of so-called masking effect. This is in contrast to ear lesions where threshold audiograms reproduced with great accuracy. These findings have led us to the routine of recording ear tones both without and with adequate masking.

Key tone audiometry is another threshold procedure. In 30% of the patients who were submitted to this test the tracings were of the type that are conventionally associated with a cochlear type of disease, thus agreeing with findings of Johnson (1968). Here again the introduction of masking may change the outcome of the test (Blegvad 1968). Usually patients with acoustic neuromas have a pure lateral sensorineural hearing loss and of a magnitude that it is appropriate to mask the contralateral normal ear. It is our impression that just this masking is a main cause of variability in the outcome of threshold test procedures that is so characteristic of these lesions. The most widely used suprathreshold procedure is the classical ABLB test which

was applied whenever possible. Here again the outcome will often be equivocal and the patient experiences great difficulty in deciding on levels that are equally loud binaurally. We agree with Simmons & Dixon (1966), that this may lead an "examiner who is naive or in a hurry to find an intensity—any intensity—which the subject will call equal to the reference ear". In some of our patients the uncertainty in this respect was so pronounced that it was irrelevant to give specific figures for the ABLB test. A positive outcome of the ABLB test indicating a cochlear lesion was found in 23%, which again is in close agreement with Johnson (1972).

Speech discrimination scores constitute another important suprathreshold measurement. This test suffers from lack of standardization and any evaluation rests on rather subjective norms for the amount of discrimination that might be expected at various levels of sensorineural loss. Even on this basis we found the test to be very valuable, especially in patients where the PTA was only moderately reduced. Here too the application of contralateral masking would often cause the performance to deteriorate considerably. The pathophysiological basis for the typical hearing loss in patients with retrocochlear disease is a reduction of the number of active fibres in the acoustic nerve and it was to be expected that abnormal findings would be present particularly in those tests that exert the greatest demand on the total transmission capacity of the nerve, in other words with the application of intense and/or prolonged sounds. The Metz test is just such a procedure and it is not surprising that it shows the highest degree of validity between all the tests. There are really no firm criteria for the evaluation of any of the tests mentioned here, this applies also to the Metz test. We used to say that a gap of more than 60 dB between the threshold for hearing and the reflex threshold signifies lack of recruitment. If this is accepted without reservation any very slight cochlear loss should be categorized as being retrocochlear.

for in essence the reflex threshold as measured in HL remains unaffected in patients with cochlear lesions. It is only when we reach magnitudes of hearing loss around 60 dB that the reflex threshold begins to increase. This is in contrast to patients with acoustic neuromas where even a very slight hearing loss will be accompanied by a very significant shift of the reflex threshold. This is particularly true for the medium to high frequencies. Sometimes there may be a reflex at 500 Hz at intensities that are not quite 60 dB above the subjective threshold. This is exemplified in our case history where we first found no recruitment and later a borderline case of recruitment.

As experience accumulates with the Metz test, it should be possible to establish a better criterion than the 60 dB gap and this will most certainly have to involve a distinction between findings at the various frequencies.

So far the general experience with the reflex decay test is quite sparse, but it should be noted that out of 5 patients with surgically confirmed neuromas, where this test was applied, 2 did not show any decay.

The absence of middle ear muscle reflexes on the tumour side in spite of a completely normal stimulus ear contralaterally has not been reported previously. There is no obvious explanation for this phenomenon, which was present in 2 patients. It seems far-fetched to suggest that it signifies an early stage of facial nerve involvement. Because of the isolated character of this symptom, a nuclear brain stem type of impairment appears more likely.

In our final conclusion, we can only agree with others that no single test suffices to distinguish cochlear from retrocochlear disease and that it is necessary to use a battery of tests. The question remains when to apply this time-consuming battery. It is important to maintain a constant awareness of the possible presence of retrocochlear disease. To us, any unexpected variability in the outcome of ordinary routine test results has gradually become one main indication to pursue the diagnosis with more elaborate procedures. It has also

been a great help always to apply the Metz test as a part of our routine clinical examination.

## ZUSAMMENFASSUNG

Die audiologischen Befunde von 125 Patienten operativ bestätigtem Acusticusneurom werden

wicklungszeit und Grösse des Tumors. Es wurde sucht den Wert folgender audiologischer Tests zu teilen: Fowler's und Metz Recruitment Test, Sp audiometrie, Tonedecay Test, Reflexdecay Test, Békésy Audiometrie. Es wird auf einige audiotop Wahrnehmungen aufmerksam gemacht, die unseres sens bisher nicht beschrieben worden sind. Im ges Krankengut kam keine normale Gehörhaftigkeit vor.

problematisch, wenn die Toenaudiogramme 100 dB oder schlechter beträgt. Deshalb haben wir u Aufmerksamkeit auf die Patienten konzentriert, die Hörverlust 80 dB oder kleiner ist. Die physiologische Ursache des typischen Hörverlust Patienten mit retrocochlearem Leiden ist eine Reduktion der Zahl aktiver Fasern im Nervus acusticus. Es

totale T beansprucht und/oder dauernden Lauten getestet wird. Gerade Metz Test dies und es kann nicht überraschen, dass er unter Tests der zuverlässigste ist. Ein einzelner Test ist nicht um zwischen cochlearem und retrocochlearem Leiden zu unterscheiden. Es ist daher erforderlich mehrere Tests zu verwenden. Jeder unerwartete Befund bei normalen Routinetests ist allmählich eine Hilfe immer den Metz Test zu verwenden.

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## A MODIFIED "SOFT SURFACE SPECIMEN TECHNIQUE" FOR EXAMINATION OF THE INNER EAR

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**Abstract** A new surface preparation method was devised for assessing intracochlear vessels and sensorineural epithelium. The method is a combination of the conventional osmic acid stained surface preparation method and a surface preparation technique with injection of Prussian blue and decalcification of the cochlea. Such a combination preserves most advantages of each and eliminates many of the disadvantages. To preserve the delicate sensory and nervous structures we examined variations of the preparation, paying particular attention to fixation, staining and decalcification procedures and solutions. Varying the preparation had a less marked effect on external wall structures than on the basilar membrane. The primary changes consisted of variation in staining contrast. The method proved useful for demonstrating the normal anatomy and pathological changes in cochlear vasculature and in sensorineural and supporting structures of the labyrinth in light and phase contrast microscopy at moderate magnifications.

We have previously reported preliminary histological observations made with a modified surface preparation technique based upon the combination of two techniques: the osmic acid staining of undecalcified temporal bones and the Prussian blue injection and subsequent decalcification of the cochlea (Axelsson et al., 1974). We have now studied a large number of cochleas from guinea pigs, chinchillas, cats and monkeys with this technique, using both normal and experimental pathological material. We report here in more detail the prepara-

tive steps and compare the present method with related ones.

### METHODS

The study of procedural variations employed 30 guinea pigs with a readily evoked Preyer reflex. In addition, 100 guinea pigs, 6 rhesus monkeys, 13 cats and 11 chinchillas were used for studies of normal cochlear vasculature.

changes, influence of noise and/or surgery, and influence of experimental acoustic tumors.

Some preparation steps were stable; others varied (Table I and Fig. 1). The basic techniques are reported elsewhere: osmic acid undecalcified surface preparation (Engström et al., 1966, Johnsson & Hawkins, 1967), Prussian blue decalcified surface preparation (Axelsson, 1968, 1972) and a modification employing both these techniques (Axelsson et al., 1974). Basically, the sequence of preparation steps included: (1) vascular contrast injection, (2) fixation, (3) osmic acid staining, (4) decalcification, and (5) slide mounting of dissected material.

#### *Perfusion and contrast injection*

Perfusion of saline and injection with Prussian blue are described in detail previously (Axelsson et al., 1974).

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## Variations (preferred technique italicized)

	Mode	Time
fixation Millonig	<i>Intracochlear inj</i>	<i>0.5 to 15 min</i>
id (0.5%) pH=6.5	<i>Immersion</i>	
aldehyde pH=5.0	<i>Intracochlear inj</i>	<i>2 to &gt;24 hours</i>
s solution pH=7.4	<i>and immersion</i>	<i>24 hours</i>
aldehyde pH=4.0	<i>Immersion</i>	
AND Nitric acid pH<1	<i>Immersion</i>	<i>as required</i>
4 H <sub>2</sub> O buffered NaOH pH=7.4		
DTA H <sub>2</sub> O not buffered, pH=5.0		
A Millonig pH=6.5		
pH<1		
AND DECALCIFICATION		
A in 2% glutaraldehyde pH=5.0	<i>Intracochlear inj</i>	<i>as required</i>
	<i>and immersion</i>	
A in Karnowsky's solution pH=6.0		
STAINING**		
(Millonig) osmic	<i>Intracochlear inj</i>	<i>3-5 min</i>
5% pH=6.5	<i>Immersion</i>	<i>5-10-15 min</i>

\* Omega Chemical Corporation Cold Spring on Hudson ■ Y  
osmic stained previously

3, 1972) Static pressure was constant as was the injection solution with to strength and temperature. Amount of ' solution varied with size of the animal: the guinea pig and chinchilla the entire system of the animal was perfused injected. In the cat and monkey the de aorta was clamped

ing vascular perfusion and removal of poral bones, the oval and round ss and the apex of the cochlea were ally opened in some of the guinea pigs. hlear structures were fixed either by injection and subsequent immersion in ve or by immersion only. Cochleas fixed with either Karnowsky's solution, acid, or glutaraldehyde, or a combina- the latter two. Using osmic acid, the leas were stained simultaneously. Some eas fixed with Karnowsky's solution

and glutaraldehyde, were decalcified, divided longitudinally and subsequently counter-stained with osmic acid.

### Decalcification

After fixation the temporal bones were decalcified. Time varied with content and strength of solution from a few hours to five days. The decalcification agent was changed daily to a fresh solution. In some guinea pigs the temporal bones were examined after simultaneous fixation and decalcification.

In all ears, following decalcification the cochlea was washed in tap water for 2 hours, dehydrated in successive stages of increasingly concentrated alcohol, and stored in glycerin. Details of the order and variations in the procedures are given in Table I and Fig. 1.

### Dissection

The dissection began with a midmodiolar or paramodiolar section made close to the round

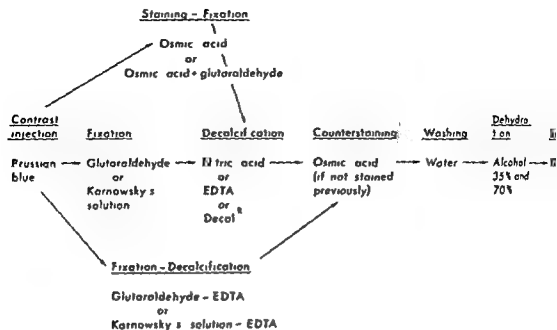


Fig 1 Procedural variations of the present soft surface specimen technique

window without affecting it. With the sectioned surface facing the microscope, the specimen was examined for such changes as distension or collapse of the vestibular membrane, occurrence of hemorrhage or debris, etc. Transverse cuts were then made with a razor blade on the apical and basal side of the spiral lamina in each turn. Spiral lamina pieces together with the attached modiolus parts were then removed by simply pulling them away from the external wall with a watchmaker's forceps. The basilar membrane of the basal turn adheres more firmly than in the other turns and must often be divided from the external wall by careful sections. The vestibular and tectorial membranes are removed from the specimen with a watchmaker's forceps stabilizing the specimen with manual pressure of a cover slip glass under stereomicroscope magnification of  $\times 50$ .

The remaining parts of the modiolus structures were removed by apico basal sections. Such pieces contain VIII nerve fibers, spiral ganglion cells, the modiolus wall, radiating arterioles, collecting venules and the artery and vein in the modiolus.

The remaining membranous external on the inside of the cochlear bone was moved with a delicate spoon shaped instrument by blunt dissection between the bone and the spiral ligament.

All dissected pieces were placed on slides in glycerin and cover slipped.

### Microscopy

In general, vasculature was studied under microscopy. sensorineuroepithelium, tectorial and vestibular membranes, vessel wall under phase contrast microscopy. The spiral lamina was examined from the inside, visualizing the peripheral vas arcades and nerve endings, and from the outside visualizing the sensory and supporting structures and the limbus vessels. The specimens are sufficiently elastic and firm they can be turned without damage.

The external wall appeared to be little affected by the variations in the preparation steps. We focused therefore, on presentation and visualization of the sensory structures of the organ of Corti.



guinea pig second turn organ of Corti longitudinal section light microscopy. After the mid-modiolar section made in the decalcified cochlea the sectioned

surface is examined before further dissection. OHC=outer hair cells TM=tectorial membrane TL=tympanic lip SL=spiral limbus

## RESULTS

### Technical Aspects

#### Injection

Previously the Prussian blue injections did not give consistently uniform vessel injection. The basal turn was injected most easily, the apical turn least well. In certain preparations some vascular tributaries were less well injected than others for no apparent reason.

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#### Fixation and staining

We obtained adequate fixation with all the methods adopted. We preferred the glutaraldehyde solution, however, because it was easier to prepare. The osmic acid procedure had two disadvantages in both staining and dissection. (1) For an even osmic stain throughout the cochlea the osmic acid has to be injected through the opened cochlea. Contrarily,

the opened cochlea showed little evidence of insufficient fixation after immersion in glutaraldehyde. (2) The time necessary for adequate fixation with osmic acid is longer than that for adequate staining of the tissue. Consequently, osmic acid must be removed from the cochlea or else the tissues stain too darkly, obscuring the vasculature. Glutaraldehyde injected following osmic acid fixation, staining both stopped further staining and also ensured adequate fixation.

A particularly fast variation of this procedure combined fixative and decalcification agent in one preparative step. However, the sensory epithelium was not as distinct with this approach. Other variations may be used to advantage for different purposes. For studies of vascular anatomy, Prussian blue injected material may be either unstained or lightly injected with osmic stain. For investigations of discrete pathological changes, windows and

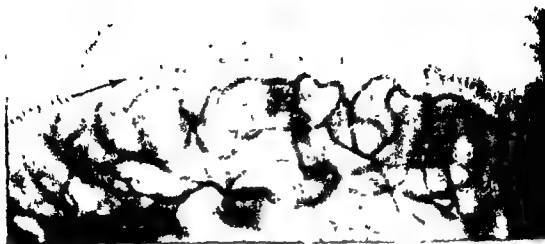


Fig. 3 Guinea pig, basal turn, spiral limbus, transverse section, light microscopy. The limbus vessels are most

easily demonstrated with an apical view row = Huschke's dendrites

apex should be opened, but injection of fixative or staining solutions should be avoided. In such cases the cochlea may be immersed in glutaraldehyde and examined after decalcification-sectioning but before counterstaining for gross changes and after osmic-counterstaining for discrete changes.

### Decalcification

Tissues decalcified in EDTA showed little significant difference from those decalcified in a weak solution (0.5%) of nitric acid. Gross distortion of the structures of the basilar membrane, evidenced by a wavy appearance of the organ of Corti, often occurred with Decal<sup>®</sup>, and sometimes with EDTA. The distortions could not be correlated with any obvious preparation variable (i.e., inadequacy of fixation, fixative pH, etc.). Definitely, best results were obtained when the decalcification solutions were changed daily.

### Counterstaining

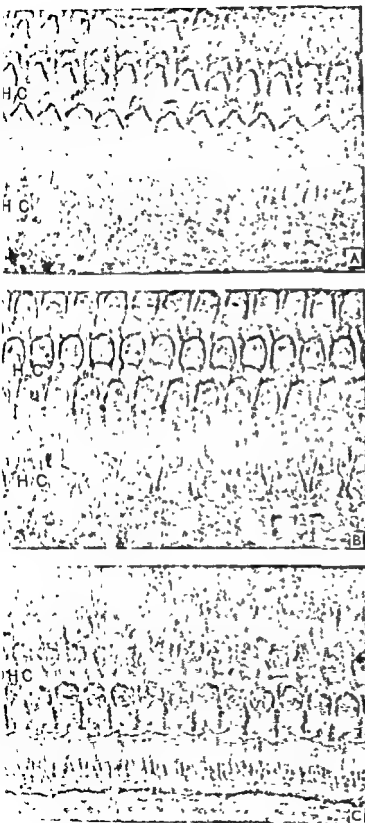
The primary precaution to be taken with osmic acid staining is control of exposure time. The tissue appears to be considerably more susceptible to overstaining when it is fresh than following fixation and decalcification. It

is difficult to escape the concern that mechanical damage to cochlear structures may be caused by direct injection of fixative or staining solutions.

Varying the preparation steps had a marked effect on external wall structures on the basilar membrane, particularly the organ of Corti. The primary changes consisted of variation in staining contrast. Many of the initially observed differences thought to be due to different preparation steps were found to be minimal as experience was gained with this approach.

### Photography

Using optical sections with phase contrast microscopy, the spiral lamina can be examined in a midmodiolar view (Fig. 2) and from apical and basal aspects (Figs. 3-5). The apical aspect provides the clearest view of the limbus vessels (Fig. 3) and limbus cells and provides a study of the stereocilia of inner and outer hair cells, the reticular membrane, pillar cells (Fig. 4), myelinated nerve fibers (Figs. 5-6) and supporting cells. The basal aspect provides clear views of the vessel of the basilar membrane (vas spirale, outer spiral ve-



*Fig 4* Guinea pig, basal turn, basilar membrane, transverse section, apical view, phase contrast microscopy. With the aid of optical sections, different structures in the organ of Corti can be visualized. (A) Stereocilia of the outer hair cells (OHC) (B) The reticular lamina as well as the inner hair cells (IHC) (C) The cell bodies of the outer hair cells, the stereocilia of the inner hair cells, and pillar cells

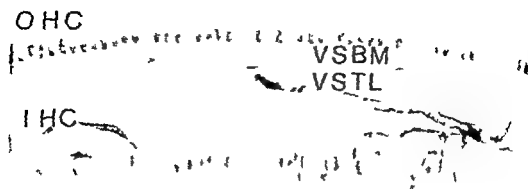


Fig 5 Guinea pig basal turn spiral lamina transverse section, light microscopy. The vessels in the spiral lamina are more clearly seen with a basal view than with an apical view. The myelinated nerve endings are clearly seen in

both views. OHC=outer hair cells, IHC=inner hair cells, VSBM=vessel of the basilar membrane, VSTL=vessel of the tympanic lip.



Fig 6 Guinea pig basal turn basilar membrane transverse section, basal view, phase contrast microscopy. Adopting high magnification and phase contrast, the vessel lumen and wall are easily visualized in relation to other

anatomical structures. OHC=outer hair cells, IHC=inner hair cells, VSBM=vessel of the basilar membrane, VSTL=vessel of the tympanic lip.



uneared pig basal turn external wall longitudinal light microscopy. The capillaries of the stria vascularis (VS) are visualized as well as the vessel(s) of the prominence (VSSP) and the collecting venules

vessel of the tympanic lip (Figs 5-6) provides a different and very clear view of eliminated nerve fibers, the radial fibers (nerve) bundles, and the nuclei in the inner ear hair cells. A fairly common finding is individual differences in 'quality' of the hair and supporting cells. In a given preparation hair cells could be quite clear, while in another preparation pillar cells were distinct, in another preparation the opposite could be found etc.

The present method permitted visualization and study of the external wall vasculature comparable to that afforded by Prussian blue or osmic staining (Fig. 7). Using different sections we viewed the vessels forming

the endolymph and perilymph and selectively studied the vessels in the deeper part of the spiral ligament. In both injected and uninjected preparations of the external wall we observed the vessel lumen, its wall, perivascular spaces, pericytes, etc. (Fig. 8), and studied the surface cells of the stria vascularis (Fig. 9). We also easily observed the structure of the vestibular and tectonal membranes. This method proved equally useful for study of the vestibular membranous labyrinth, including both vasculature and neural structures (Figs 10-12).

We have used the present method to study discrete pathological changes in membranes and lymphatics, in hair cells, nerve endings, vasculature and supporting structures that resulted from mechanical and electrolytic lesions of the cochlea (Hallen et al., 1974a, b), from noise exposure (Lipscomb et al., 1974), and from middle ear pressure changes (Lamkin et al., 1974).

## DISCUSSION

Five principal methods have traditionally been used to visualize intracochlear structures:

Serial sections after conventional staining, examining the longitudinal section (e.g. Guild, 1919, 1932, 1937; Schuknecht 1953, 1959; Ward & Lindsay, 1964).

Surface techniques with staining of the intraluminal blood (e.g. Scuderi & del Bo, 1952; Smith, 1954; Maass, 1969).

Surface techniques with staining of the vascular wall (e.g. Smith, 1951; Nomura & Hiraide, 1968; Kimkac et al., 1969; Sugar et al., 1972).

Surface techniques with osmic staining (e.g. Retzius, 1882; Neubert, 1950; Engstrom et al., 1963, 1966; Spoendlin, 1966; Johnsson & Hawkins, 1967; Kirchner, 1968; Bohne, 1972).

Surface techniques with injection of different contrast media or intracochlear precipitation of stain (e.g. Eichler, 1892; Siebenmann, 1894; Naheysa, 1923; Smith, 1951, 1953, 1954,





Charachon, 1961, Axelsson 1968, 1970, Hansen & Mazzoni 1970)

Most of these methods have been relation to possible decalcification embedding of the material and type calcification and embedding agent been assessed by light and phase and lately by electron and scanning scopy. Sometimes all five of these have been adopted on the same

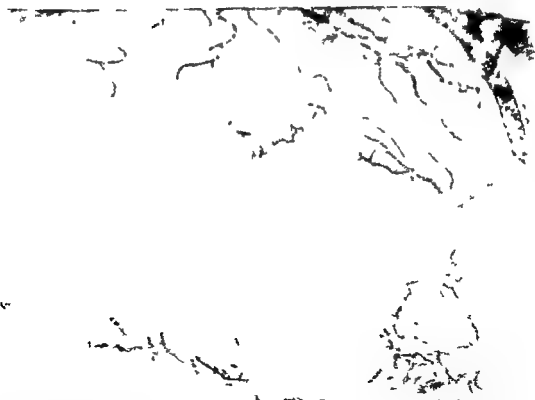
The present method is a surface method introduced by Retzius (forgotten and revived by several recent decades (Neubert 1950, E al 1966, Johnsson & Hawkins). Cochlear vasculature is visualized by of a contrast material. Prussian sensorineurepithelium is stained by acid and the temporal bones are. Consequently, combination of the currently much used (undecalcified) osmic staining technique, the 'soft surface (decalcified) Prussian'



Fig. 8 Guinea pig basal turn scala vestibuli longitudinal section phase contrast microscopy. In the contrast injected preparation (A) the occurrence of avascular channels (AVC), perivascular spaces (PVS) and pericytes (PC) as well as the vessel wall can be studied. In the contrast injected preparation (B) under these high magnification, these features may be studied in addition to the more adequate visualization of the vessel lumen.



9



10

9 Guinea pig basal turn stria vascularis longitudinal section phase contrast microscopy. In preparations with unsuccessful contrast injection on the vessels and their content are visualized in relation to the surface cells

Fig 10 Guinea pig macula sacculi vessels injected by contrast are visualized in relation to the nerve fibers



Fig 11 Guinea pig posterior ampulla. The vasculature of the ampulla wall and the canal are visualized in relation to the crista (arrow).

surface technique. Table II is a list of the principal advantages and disadvantages that we see in each of the surface preparation procedures.

We suggest, at least in some regions, an almost cavalier attitude in this approach: tissue is quite resistant to damage and handled, turned and coverslipped freely.

Table II Comparison of the surface preparation techniques

Prussian blue decalcification	Hard surface preparation technique (osmic only)	Soft surface preparation technique (Prussian blue plus osmic)
<b>Advantages</b> Fast Easy dissection High contrast Whole cochlea assessed Vasculature better than osmic Good for anatomy	<b>Advantages</b> Vessel wall and lumen assessed Sensory and nervous structures seen at high magnification Good for pathology	<b>Advantages</b> Fast Easy dissection Whole cochlea assessed Good for anatomical and pathological studies Sensory nervous and vascular structures can be examined at low and high magnification
<b>Disadvantages</b> Crude Sensorineuroepithelium less well visualized Vessel wall lumen artificially injected Less good for pathology	<b>Disadvantages</b> More time-consuming Bone chips Not all parts seen Deficient contrast Less good for anatomy	<b>Disadvantages</b> Vessel lumen artificially injected



Guinea pig crista ampullaris. The rich vascular network is visualized through the surface epithelium.

Early, few steps are sacred in this technique. Fresh solutions carefully prepared (of course important, however, variations in different steps and the elimination of steps may be suggested as useful modifications in some experiments. For study of the ear vasculature we suggest the procedure employs Prussian blue only. If the vessels are to be studied in relationship to supporting structures we suggest a light osmic acid staining by injection. On the other hand to investigate possible discrete pathological changes we recommend immersion of the cochlea with opened windows in glutaraldehyde. Intracochlear structures may be counterstained with osmic acid after decalcification with this modification.

This method proved useful for study of the cochlear vasculature and sensorineuroepithelium of the cochlea as well as the vestibular end organ. It was also useful in our studies of experimentally induced changes on both vasculature and sensorineuroepithelium (induced by

electrolytic and mechanical lesions of the external wall, middle ear pressure changes and influence of noise and/or salicylate, etc.). We would further suggest that it may be useful for studying many other and different experimentally induced effects on cochlear vasculature and sensorineuroepithelium which give substantial cochlear changes observable with light and phase contrast microscopy and in magnifications ranging from  $\times 10$  to  $\times 100$ . We have yet to evaluate the usefulness of this method for electronmicroscopic studies.

## ZUSAMMENFASSUNG

Eine neue Methode zur Untersuchung der intracochleären Strukturen sowie der Gefäße und des Sensorineuroepithels wird dargestellt. Die Methode ist eine Kombination der allgemein angewandten Osmiumsäuremethode mit gefärbten Häutchenpräparaten in einer Methode bei der Berlinerblau injiziert das Felle beim entkalzt und die Cochlea wiederum Häutchenpräparaten studiert wird. Die Kombination dieser zwei Methoden bewahrt die Vorteile und eliminiert viele der Nachteile jeder einzelnen Methode. Die verschiedenen Abschnitte der Technik werden detailliert

beschrieben und es werden die Variationen in verschiedenen Abschnitten bewertet. Ausserdem wird eine photographische Dokumentation der Befunde der neuen Methode im normalen und pathologisch veränderten Innenohr vorgelegt.

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## SPEECH DISCRIMINATION IN QUIET AND IN WHITE NOISE BY PATIENTS WITH PERIPHERAL AND CENTRAL LESIONS

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Speech-discrimination scores in quiet and in noise (0 dB S/N ratio) were obtained from six groups of patients. Differences of 40% or more between scores in quiet and in noise were observed for less than 1% of all ears tested but were found for 8.0% of ears with trauma, 48% of ears with Meniere's disease, 48% of ears with subsequently surgically confirmed tumors, 14% of ears of patients with multiple sclerosis and 42% of ears contralateral to the lesion in patients with temporal lobe damage.

Persons with sensorineural hearing loss have difficulty in understanding speech during quiet (Simonton & Hedgecock 1953; Palva 1965; Liden 1967; Olsen & Ross et al 1965; Tillman et al 1970; Cooper & Keith & Talis 1971; Shapiro et al 1972; Jokinen 1973). The most common finding in studies of speech discrimination in the presence of white noise is the variability among subjects. While speech-discrimination scores in quiet have been uniformly high for persons with normal hearing, scores in the presence of noise have varied widely in every investigation. Usually, the variability in performance has been even greater for persons with impaired hearing. These variability differences between scores obtained in quiet and in noise have been greater for persons with sensorineural (presum-

ably cochlear) hearing losses than for persons with normal hearing.

Some researchers have investigated the potential of speech in white noise as a diagnostic indicator of dysfunction at various segments of the auditory nervous system. In 1972, Katinsky & Lovrinic reported severe reduction in speech discrimination in white noise from ears of patients who were subsequently found to have 8th nerve tumors.

Dayal et al (1966) indicated that speech discrimination in the presence of white noise also can be abnormal in patients with brain stem involvement. They reported that some patients with multiple sclerosis had speech discrimination losses in white noise that were not expected on the basis of their pure tone test results. Similar findings were described recently by Morales Garcia & Poole (1972) for 10 patients with brain stem tumors and 5 patients with multiple sclerosis and by Noffsinger et al (1972) for a larger sample of patients with multiple sclerosis.

Sinha (1959) was the first to report results obtained from the use of monosyllables presented in white noise in the evaluation of patients who had cortical lesions. When compared to normal subjects, patients with temporal lobe involvement had decreased performance on speech discrimination in white noise in the ear contralateral to the lesion. Patients with cortical lesions that did not affect

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Table 1 Speech discrimination study of six groups of subjects

Group	Sample size	Age years	
		Mean	Range
Normal	75	36.9	13-67
Noise trauma	25	42.2	22-52
Meniere's disease	25	53.1	16-74
8th nerve tumor	21	42.5	17-65
Multiple sclerosis	31	37.8	20-59
Temporal lobe lesion	24	36.7	18-65

the temporal lobes had scores equal to those of the normal subjects. Sinha's observations were supported by the publications of Morales Garcia & Poole (1972) and Heilman et al (1973).

There is no doubt that discrimination of monosyllables can be markedly decreased by lesions in the cochlea as well as by lesions at nearly every segment of the auditory nervous system from the 8th nerve to the temporal lobe. However, no previous study has compared speech discrimination performance in quiet and in white noise for patients with cochlear, 8th nerve, brain stem or cortical lesions utilizing a single test and procedure.

### PROCEDURE

Routine air-conduction and bone conduction audiometry, speech reception threshold and speech discrimination tests were completed individually for all subjects in a double walled test chamber. The test equipment was calibrated routinely and maintained to comply with ANSI 1969 specifications (American National Standards Institute 1970). Tape recorded materials were used for all speech tests. Speech reception thresholds were determined with spondaic words employing the descending method described by Tillman & Olsen (1973). Speech discrimination was assessed at 40 dB sensation level re the speech reception threshold utilizing Northwestern University Auditory Test No. 6 (Tillman & Carhart 1966) in quiet and in white noise at 0 dB signal/noise (S/N) ratio. The speech signal and noise were presented via a speech

audiometer (Grason Stadler 162). The opposite ear was masked for bone-conduction testing and for the air-conduction pure tone and speech tests as needed. A separate noise source was used to mask the opposite when this was necessary during the speech white noise test.

### SUBJECTS (Table 1)

A group of 75 subjects (150 ears) with no apparent disease nor significant hearing impairment was tested to provide normative data against which the performance of the patients with peripheral auditory disease or central nervous system involvement were compared. These normal subjects had pure tone thresholds from 125 to 8000 Hz, no poorer than 20 dB HL (re ANSI 1969) at any frequency, pure tone averages (500 to 2000 Hz) speech reception thresholds of 25 dB HL (ANSI 1969) or better bilaterally, their speech discrimination scores were 90% or better in quiet at 40 dB SL. The mean age for these subjects was 36.9 years and the ages ranged from 13 to 62 years. None was a sophisticated or practiced listener.

The other groups consisted of patients with medically diagnosed hearing losses due to noise trauma, Meniere's disease or 8th nerve tumors (subsequently surgically confirmed) and patients with lesions of the central nervous system. Patients with central nervous system involvement were separated into three groups: patients with multiple sclerosis, patients who had documented temporal lobe involvement due to cerebrovascular accident or who had undergone neurosurgery for removal from intractable seizures.

Twenty five patients had noise trauma. Their pure tone averages and speech reception thresholds were within normal limits (25 dB HL or better) but each had characteristic audiometric configuration associated with noise induced or acoustic trauma hearing impairments that is a high frequency notch of reduced sensitivity that was deepest at 1000, 4000 or 6000 Hz.

## 1 Pure tone and speech results for six groups of subjects

	No of ears	Pure tone (dB HL)		Speech reception threshold (dB HL)		Speech discrimination (%)					
		Mean	Range	Mean	Range	Quiet		White noise		Difference*	
						Mean	Range	Mean	Range	Mean	Range
normal	150	6.5	-7 to 21	3.8	-5 to 21	98.2	92 to 100	73.8	56 to 94	24.4	6 to 44
deafness	50	11.0	0 to 23	6.0	-5 to 18	97.2	90 to 100	69.3	52 to 88	27.9	4 to 44
tumor	25	42.1	27 to 62	38.1	15 to 64	85.4	60 to 100	44.6	0 to 72	40.8	18 to 82
sclerosis	21	25.9	10 to 48	22.2	-7 to 45	84.0	56 to 100	36.8	0 to 74	47.2	20 to 92
deaf	50	8.4	-2 to 18	5.0	-6 to 18	97.6	92 to 100	70.7	36 to 92	26.9	6 to 54
total	24	11.9	0 to 32	10.4	0 to 30	96.3	84 to 100	53.2	8 to 78	43.1	22 to 84

\*us white noise scores

hearing sensitivity at 8000 Hz. This was common to both ears of all these. Hence, there were 50 ears in the trauma sample. Speech discrimination in as 90% or better for all of them at 40 dB.

nly five patients had a medical diagnosis of unilateral Meniere's disease, this consisted of 25 ears. Various degrees of low frequency hearing loss associated with Meniere's disease were present at the time of testing, as reflected by the range of pure tone averages (27 to 62 dB HL) and speech discrimination scores in quiet (60 to 100%) (Table II).

nly one patient subsequently found to have a unilateral 8th nerve tumor (surgically removed) was tested. (Only the results for impaired ears are given in Table II.) These ears demonstrated hearing sensitivity ranging from normal to mild hearing impairment for speech and speech discrimination in quiet, ranging from 56 to 100%.

nly one patient had confirmed multiple sclerosis. The ears of these patients were found to have 125 to 8000 Hz pure tone averages within normal limits (25 dB HL) or better, a speech reception threshold of 25 dB HL, and speech discrimination of 90% or better. These criteria were imposed in order to eliminate possible influences of central nervous system lesions associated with multiple sclerosis on speech discrimination in white

noise without the contaminating effects of a loss in hearing sensitivity attributable to other involvements. Both ears of 19 of the patients and one ear of each of the remaining 12 patients met these criteria.

Of 24 patients with temporal lobe lesions, three had had hemispherectomies when they were young in order to overcome serious convulsive disorders. The left cortical hemisphere had been removed in one patient, and a right hemispherectomy had been performed in the other two. Eleven patients had undergone partial temporal lobectomies (six left, five right) to overcome seizures. The other 10 patients had suffered cerebrovascular accidents that affected the temporal lobe. The damage had occurred in the left temporal lobe in six patients and in the right temporal lobe in four. The ears contralateral to the damaged hemisphere were included in this sample. These ears were characterized by essentially normal speech reception thresholds and good speech discrimination in quiet (84 to 100%) (Table II).

Each subject tested in this project was capable of communicating with the audiologist and of understanding and following test instructions. All were able to respond reliably to the various auditory tests administered.

## RESULTS

None of the 150 ears in the normal hearing group had pure tone averages or speech



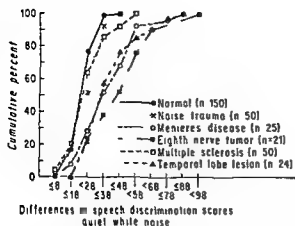


Fig. 1 Cumulative frequency distribution of speech-discrimination scores (quiet minus white noise) for six groups of subjects, *n*, number of ears in each group

reception thresholds poorer than 21 dB HL, and all had speech-discrimination scores in quiet of 92% or better (Table II). When the monosyllables were mixed with white noise at a S/N ratio of 0 dB, the average score was almost 74%, the range was from 56 to 94%. The mean difference between scores in quiet and in white noise was about 24% and ranged from 6 to 44%.

The mean score in quiet for the 50 ears in the group with noise trauma was similar to that of the normal group. Speech discrimination in noise, however, was about 4% poorer on the average than for the normal group. The range of performance in noise was about the same for both groups.

As expected, the 25 patients with unilateral Meniere's disease had average thresholds and speech-discrimination scores in quiet that

were poorer than those of the group with trauma. In addition, their breakdown of performance in white noise was also greater: 40.8% as compared to 27.9% for the group with noise trauma. The range of difference (quiet minus white noise scores) was larger for the group with Meniere's disease (18 to 88%) than for the group with noise trauma (18 to 88%).

Even though the 21 ears with 8th nerve tumors generally had better hearing sensitivity than did the ears with Meniere's disease, had speech-discrimination scores in quiet that were equal to those of the group with Meniere's disease, their average performance in white noise was about 8% poorer.

The performance of the group with multiple sclerosis was similar to that of the normal hearing group and the group with noise trauma. However, the scores in white noise obtained for some of the patients with multiple sclerosis were poorer than scores for a subset of the normal subjects, although both groups had normal hearing sensitivity and speech discrimination in quiet.

The group with temporal lobe lesions had essentially normal sensitivity and speech discrimination in quiet for the ears contralateral to the cortical lesion. Only one patient had hearing sensitivity poorer than 25 dB HL and speech discrimination below 90% in quiet. However, the average difference between their scores in quiet and in white noise was about 24%, which was large as that observed for the group with Meniere's disease and the group with 8th nerve tumors, although the group with temporal lobe lesions had better hearing sensitivity and speech discrimination in quiet.

Table III Incidence (in per cent) of differences in speech-discrimination scores for six groups of subjects

Group	Difference scores (quiet minus white noise scores)									
	0-8	10-18	20-28	30-38	40-48	50-58	60-68	70-78	80-88	90-100
Normal	0.7	16.0	60.6	22.0	0.7					
Noise trauma	2.0	10.0	40.0	40.0	8.0					
Meniere's disease		8.0	20.0	24.0	16.0	24.0		4.0	4.0	4.8
8th nerve tumor			23.8	14.3	14.3	23.8	14.3	4.8		
Multiple sclerosis	4.0	16.0	44.0	22.0	6.0	8.0				
Temporal lobe lesion			22.8	35.0	19.2	7.7	3.8	7.7	3.8	

#### IV Pure-tone and speech results for groups of patients with cortical lesions<sup>a</sup>

	Group			
	Temporal lobectomy (11 patients)		Cerebrovascular accident (10 patients)	
	Mean	Range	Mean	Range
tone (dB HL)	6.9	0-20	19.7	11-21
reception				
word (dB HL)	6.8	0-15	16.2	9-30
(%)	99.2	92-100	93.2	84-100
noise (%)	68.9	62-78	35.4	8-54
noise (%) <sup>b</sup>	30.3	22-38	57.8	36-84

<sup>a</sup> contralateral to lesion

<sup>b</sup> minus white noise test scores

st of the normal ears had scores in noise were 20 to 28% poorer than their performance in quiet (Fig 1 and Table III) of the remainder achieved scores that 10 to 18% or 30 to 38% poorer. Only two varied from this pattern, one attaining a that was reduced from the score in quiet % and one by 44%. These data indicate a difference of 40% or more between b-discrimination scores in quiet and in noise from these test materials is unfavourable for persons with normal hearing. There differences of 40% or greater were considered abnormal for the purposes of this

the distribution of quiet white noise differences for the group with noise trauma was similar to that of the normal group, but there a slight skewing of the distribution to the left (Fig 1). There were four ears (8%) in this group whose scores were reduced by 40% or more. The incidence of marked reduction in speech understanding in white noise was considerably greater for the group with Meniere's disease: about one-half of the patients in this group attained scores in white noise that were reduced by 40% or more from their scores in quiet. The distribution for the group with Meniere's disease was different from that of

the normal group or the group with noise trauma.

Quiet-white noise differences exceeding 38% was most frequent in the group with 8th nerve tumors. The decrease in speech discrimination performance in white noise was most commonly from 40 to 68%, one patient had a score of 92% in quiet but was unable to understand even one monosyllable in white noise at a 0 dB S/N ratio. The cumulative distribution curve for this group paralleled the curve for the group with Meniere's disease but was shifted somewhat to the right throughout (Fig 1).

While most patients with multiple sclerosis did not have inordinate difficulties in understanding speech in white noise, seven (14%) had performances decreased by 40% or more by the addition of white noise. Decreases this large were unilateral for five patients and bilateral for one patient. This incidence of excess breakdown in speech discrimination in white noise is striking when one considers that each of these subjects was required to have normal hearing sensitivity and speech discrimination in quiet in order to be included in the study. The lower part of the cumulative distribution curve for this group followed or fell between the curves for the normal group and the group with trauma but then fell below these groups in the upper portion (Fig 1).

A higher incidence of excess decrease in

Table V Incidence (in percent) of differences in speech discrimination scores for two groups of patients with cortical lesions

Difference scores (quiet minus white noise scores)	Group	
	Temporal lobectomy (11 patients)	Cerebrovascular accident (10 patients)
20-28	45.5	0.0
30-38	54.5	10.0
40-48	0.0	30.0
50-58	0.0	20.0
60-68	0.0	10.0
70-78	0.0	20.0
80-88	0.0	10.0

understanding speech in white noise was observed for the contralateral ears of patients with temporal lobe lesions. Approximately two-fifths of these patients had decreases of 40% or more in speech discrimination performance when white noise was mixed with the speech signal. Curiously, the cumulative distribution curve for this group paralleled most closely the curve for the group with Meniere's disease in spite of no loss in hearing sensitivity for the group of patients with temporal lobe lesions. For all 24 of these patients, scores in white noise in the ipsilateral ear were reduced no more than 38% from their optimal performance in quiet, the mean decrease was 28.4%, and the range was 16 to 38%.

### DISCUSSION

Our data support the rather marked variability in the performance of subjects with normal hearing in speech discrimination in noise that has been noted by others. The range of differences in quiet white noise scores obtained in our study was similar to that reported by Keith & Talis (1972) but was somewhat less than that shown by Morales Garcia & Poole (1972) for similar 0 dB S/N ratio conditions. The rather sharp delineation in performance observed in our study (with only one normal ear yielding a difference greater than 38% between scores in quiet and in white noise) is encouraging and suggests that a difference of 40% or greater can be considered abnormal when using the test materials utilized in this study. However, clinicians who wish to utilize a speech in noise test must gather normative data with the specific test materials they use. Some investigators have employed more favorable S/N ratios in order to obtain scores sufficiently above 0% to observe meaningful differences in performance. Furthermore, consideration of the range and distribution of performance by a group of normals is most important if a test is to be utilized clinically.

The data obtained in our study for the group

with noise trauma (with the associated high frequency loss) and the group with Meniere's disease (generally with low frequency or hearing impairments) are in agreement with the findings of Ross et al (1965) and Keith & Talis (1972). They noted that speech discrimination performance in white noise was severely affected by low frequency sensorineural hearing impairments. The results of their studies, however, cannot be directly related to findings from our study because they did not report the cause of the hearing impairments. Nevertheless, there is agreement among the studies of Ross et al (1965) and Keith & Talis (1972) and the present study that low frequency hearing losses generally decrease speech discrimination performance in white noise more severely than do high frequency losses when the impairments are attributable (presumably) to cochlear involvement.

As suggested by Katinsky & Lov (1972), patients with 8th nerve tumors have excess reduction in speech discrimination in white noise. The data from our study, however, indicate that considerable caution must be attached to such an assertion. In our study, eight patients with 8th nerve tumors had scores in quiet and in white noise that differed by less than 40%. Second, our data indicate that other auditory involvement also can result in a marked reduction in speech discrimination performance in white noise.

Observation of differences of 40% or more in white noise via the contralateral ear has been documented earlier (Sinha & multiple sclerosis supports previous reports by Dayal et al (1966), Morales Garcia & Poole (1972) and Noffsinger et al (1972). In the present study, six of the 31 patients with multiple sclerosis revealed excess reduction in speech discrimination in white noise (three unilaterally and one bilaterally) in spite of normal hearing sensitivity for all audiotaped test frequencies and excellent speech discrimination in quiet. Neurologic symptoms in these six patients varied within the cer-

ous system, and there was no obvious onship between the breakdown in white and sidedness, bilaterality, or inferred of lesion. Morales Garcia & Poole (1972) similar conclusions

■ difficulty encountered by patients with oral lobe lesions in understanding speech hite noise via the contralateral ear also been documented earlier (Sinha 1959, ales Garcia & Poole, 1972, Heilman et al . ) However, consideration of data ob d from the patients with temporal lobe ns in our study revealed two distinct ps

rformance of the 11 patients who had rgone partial temporal lobectomy for in able seizures was within normal limits les IV and V). Of the 10 patients who cerebrovascular accidents, only one (left brovascular accident) showed normal ormance on the speech in white noise test other nine patients had greater difficulty this task showing differences of 42 to between their scores in quiet and in white ■ (Table IV). Of three patients with ispheredectomy two revealed excess break n in speech discrimination in white noise right one left) whereas the third (right ispheredectomy) did not

ndings for the patients with temporal ctomy contrast with the results of Sinha 9) and of Heilman et al (1973) who noted eased performance for the ear contra ral to the lesion compared to performance the ipsilateral ear. Such differences be en ears were not observed for the patients n temporal lobectomy in our study in that mean decrease for the ipsilateral ear was 3% compared to 30-4% for the contra ral ear of these patients. This result was ained even though as much as 52 mm of the enior portion of the left temporal lobe or 70 of the anterior portion of the right pporal lobe were removed in individual ients

From these results it would appear that the mage either involved portions other than the

anterior segment or involved more than the anterior portion of the temporal lobe for the patients with cerebrovascular accident. This statement is based on the observation that all but one of these patients demonstrated excess difficulty on the speech in the white noise test. Of course, for the patients with hemispheredectomy, the entire temporal lobe had been removed, two of these revealed differences of 46 and 48% between their scores in quiet and in white noise, but one scored only 32% poorer in noise than in quiet.

The difference in performance via the contralateral and ipsilateral ears is an important consideration in patients with cortical lesions. The observation that, when excess difficulty on the speech in white noise was found, it was always for the ear contralateral to the lesion regardless of whether the lesion was in the right or in the left temporal lobe, is in agreement with the findings of the previously cited investigators. Such findings indicate that the right temporal lobe is also involved in processing or transmitting (or both) speech signals even though the left temporal lobe is generally considered to be the dominant language center in man. These comments are consistent with the conclusions reached by Sparks et al (1970) and Berlin et al (1972) based on their findings from dichotic speech tests of patients with temporal lobe lesions. It also demonstrates the dominance of the contralateral auditory tract in carrying information to the temporal lobe.

From the preceding discussion it is apparent that lesions at any point in the auditory system from the cochlea through the temporal lobe can result in marked reduction in speech discrimination in white noise. In patients with cochlear involvements low frequency hearing impairments apparently disturb speech discrimination in white noise more than do cochlear lesions resulting in high frequency losses. However large differences between speech discrimination scores attained in quiet and in white noise in conjunction with normal hearing sensitivity or even a predominantly high

frequency hearing loss probably reflect neural involvement of some portion of the auditory system. Thus, results from speech in the white noise test may have some clinical usefulness in revealing abnormalities in auditory function but not in suggesting a particular site of involvement as being responsible for the dysfunction.

## ZUSAMMENFASSUNG

Die Ergebnisse der Sprachdiskrimination im Stille und im Weissen Lärm (Verhältnis 0 dB S/N) von sechs Versuchsgruppen wurden untersucht. Bei den Ergebnissen im Stille und im weissen Lärm wurden Unterschiede von 40% oder mehr bei weniger als 1% der geprüften normalen Ohren beobachtet. Diese Unterschiede wurden aber bei folgenden Gruppen festgestellt: bei 80% der Ohren mit Lärm-Trauma, bei 48% der Ohren mit Meniereschem Syndrom, bei 62% der Ohren mit nachträglich aufgrund chirurgischer Eingriffe festgestellten VIII Nerv Tumoren, bei 14% der Ohren von Patienten mit multipler Sklerose und bei 42% der den Temporalappenverletzungen gegenüberliegenden Ohren von gehirn geschädigten Patienten.

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## UNDISTORTED AND FILTERED SPEECH AUDIOMETRY IN CHILDREN WITH NORMAL HEARING

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act Undistorted and filtered speech tests were carried out in 140 test subjects with normal hearing acuity ranging in age from 4 to 19 years. Routine adult word lists were used in all tests. The intelligibility of undistorted speech rose markedly in the age groups from 4 to 9 years, correspondingly in the filtered speech test up to 11 years, taking the group 16-19 years as basis of reference. According to these results a speech discrimination test can be made in childhood with the same methods as in adults. If the results of the binaural test are taken into account, in the filtered speech test the binaural test result and the monaural discrimination of the left ear were significantly better than the binaural test results for the right ear up to the age of 12-13 years.

Speech discrimination tests can be carried out from preschool age by using the ordinary tests prepared for adults, utilizing the standards in different age groups. Even distorted speech tests have been utilized in children (Boothroyd, 1969). Using low pass and high pass filtering, speech discrimination scores in children were noticeably lower than those of adults. High pass filtering had a more pronounced effect on the scores in low pass filtering on the scores in children. Maximal discrimination of normal hearing children increased significantly between the ages of 8 and 9 1/2 years (Boothroyd, 1969). Siegenthaler (1969) supposed that the auditory functions have stabilized at the adult level at the age of 12 years and in most cases even at 8 years.

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To get a good reference score for speech audiometry in children using the same methods as for adults, we performed undistorted and filtered speech discrimination tests in a group of normal children.

### MATERIAL AND METHOD

The study consisted of 140 test subjects, 78 girls and 62 boys, ranging in age from 4 to 19 years. There were 20 subjects in each of the age groups of 4-5 years, 6-7 years, 8-9 years, 10-11 years, 12-13 years, 14-15 years and 16-19 years.

Routine clinical otorhinolaryngological examination was made in each case. None of the test subjects had any ear diseases.

Pure tone threshold measurements by air and bone conduction were made at 125, 250, 500, 1000, 2000, 4000, 6000 and 8000 Hz with a Madsen Model OB 60 audiometer equipped with Beltone TDH 39 earphones. Calibration of the audiometer was made according to the ISO standard.

Undistorted speech audiometry was carried out by the method of T. Palva (1952), measuring the speech reception threshold (SRT) and the discrimination of speech 30 dB above the SRT. The same vocabulary was used as in routine adult tests. A Madsen Model SU 20 speech unit was connected to the system used in pure tone audiometry.

Table I Average pure tone thresholds in different age groups

Age group		Frequency (Hz)							
		125		250		500		1000	
		Mean	S D	Mean	S D	Mean	S D	Mean	S D
4-5	right	14.0	5.0	11.2	5.1	8.7*	3.9	8.5**	2.9
	left	13.7	4.5	11.0	4.8	9.0*	4.5	8.0*	4.1
6-7	right	12.2	4.7	10.0	5.1	7.7	4.4	6.2	4.2
	left	11.7	5.4	9.0	5.8	7.2	4.1	4.7	4.4
16-19	right	11.2	3.6	8.7	2.7	5.5	2.8	4.2	2.4
	left	9.5	2.2	8.0	2.5	5.7	2.9	4.5	3.2

Differences were calculated relative to the oldest age group

Significance level  $p \leq 0.01 = *$   $p \leq 0.001 = **$

The filtered speech test was carried out by the method of A. Palva (1965). Two bands of speech, 480-720 Hz and 1800-2400 Hz, were used. Each band alone gives the same discrimination of about 15-20%. When the bands are presented together monaurally or binaurally, each band to either ear, the level of discrimination rises to about 80% in normal young people of 20-25 years.

Word lists are used so that the test words are presented to the subject automatically in the following order. The first word is given to the right ear on both bands, the second to the left ear on both bands, the third word binaurally one band to the right ear and the other to the left, the fourth word in a similar manner to the first and so on. Each test, on the right ear, on the left ear and binaurally, is carried out using 90 words. One sensation level of 50 dB is used. Cross hearing is eliminated by using insert type earphones. The test yields three discrimi-

nation percentages, one for the right ear, one for the left ear and a binaural one.

The statistical analyses were performed at the Computer Centre of the University of Helsinki. The averages and their standard deviations were calculated. Comparison of two groups was made by applying the Student's *t*-test. A difference is termed significant if the corresponding value of probability ( $p$ ) is

## RESULTS

The pure tone thresholds of the two youngest age groups and the oldest group are shown in Table I. The averages for both ears were calculated separately in each group and for each frequency. No significant difference between the right and left ear was obtained for any frequency.

For the group 4-5 years the average thresholds for 1000 and 2000 Hz and the group 6-7

Table II Discrimination scores in undistorted speech audiometry

	Age group													
	4-5		6-7		8-9		10-11		12-13		14-15		16	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	
Right	83.3**	6.9	89.1**	6.1	90.1**	5.2	95.2	5.0	96.5	3.7	96.4	5.5	98.1	
Left	82.8**	7.4	88.5**	6.6	90.6**	5.9	92.6	5.0	97.2	3.0	97.1	3.9	96.1	

Differences were calculated relative to the oldest age group

Significance level  $p \leq 0.01 = *$   $p \leq 0.001 = **$

S D	6 000		8 000	
	Mean	S D	Mean	S D
34	8.2	4.4	10.2	7.3
50	9.7	6.6	11.2	8.9
51	9.7	5.2	8.5	5.1
47	8.0	5.7	8.5	6.5
39	7.5	8.8	10.0	8.4
45	8.2	7.7	8.5	8.7

Hz from the oldest group. The pure tone holds in all other age groups were the same as the oldest group.

Results of the undistorted speech test are given in Table II. Discrimination of undistorted speech in the three youngest age groups was significantly poorer than in the oldest group, but the loss of discrimination was only 5%. There was no significant difference between the right and the left ear in any of the groups.

Table III and Fig. 1 shows the results of the distorted speech test. Discrimination was found to gradually increase with age. The three youngest age groups differed significantly from the oldest group as regards all three test objects. The difference in discrimination between groups 10-11 years and 10-19 years was significant in the right ear and in the binaural. In the youngest age group the discrimination values were about 40% lower than in the oldest group. Individual variations in results were slightly larger in the three youngest age groups.

The differences in discrimination scores for left-ear, right ear and binaural hearing were calculated in each individual case and the results treated statistically (Table IV). Discrimination was significantly better in the left ear than in the right in the groups 4-5 years, 6-7 years, 10-11 years and 12-13 years. Binaural discrimination was significantly better than the figure for the right ear in the groups 4-5 years and 10-11 years. Discrimination in the left ear did not differ significantly from the binaural result in any one of the groups.

Differences in discrimination between the left and right ear in the monaural test are given in Fig. 2. Asymmetry in discrimination score exceeding 15% was found in the groups 10-11 years in 2 cases (10%), 12-13 years in 1 case (5%), and 16-19 years in 2 cases (10%). In the oldest age group the difference was less than 5% in 70% of cases, while in the three youngest groups the corresponding figures were 35%, 40% and 60%. Binaural discrimination was in no case more than 10% inferior to the result for the poorer ear in the monaural test. Thus a positive binaural test was obtained in none of the groups.

## DISCUSSION

Our results as regards pure tone thresholds agree with those reported in earlier studies (Eagles et al., 1963; Siegenthaler, 1969; Fiori, 1972). There was a significant increase in hearing acuity over the age range 4-7 years while from 8 years upwards none of the groups differed significantly from the oldest age group.

### Table III Discrimination scores in filtered speech test

Age group		4-5		6-7		8-9		10-11		12-13		14-15		16-19	
trial	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	
	41.3**	13.2	54.8**	10.0	66.4**	14.0	73.0*	7.9	78.8	11.0	82.9	7.7	82.4	10.8	
	46.7**	10.9	58.9**	10.4	68.6**	11.2	79.2	7.6	83.9	9.1	85.7	6.8	85.4	11.3	
	46.3**	12.5	56.5**	11.4	68.6**	13.8	78.1*	6.6	81.9	11.3	84.5	7.2	85.2	8.3	

\* Scores were calculated relative to the oldest age group.  
 † Chance level:  $p < 0.01 = *$   $p < 0.001 = **$



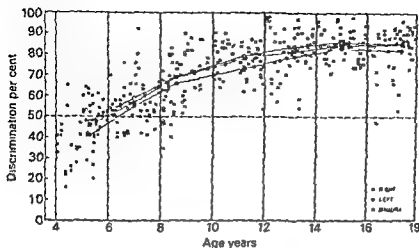


Fig. 1 Effect of age on filtered speech test

For speech audiometric studies we used the same word lists as in adults. Discrimination scores in the nondistorted speech test increased significantly in the age range from 4 to 9 years compared with the oldest age group. In the age group 4-5 years the discrimination score was about 15% lower than in the oldest age group whereas in the group 10-11 years the score was only 3% lower.

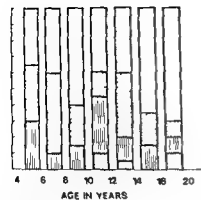
According to Boothroyd (1970) the relatively lower scores of younger normally hearing children may be due to the fact that the child may not have a complete set of phonemic categories or his vocabulary may not include all the test words. Furthermore the ability to carry out the decision process may be imperfectly developed and developmental articulation difficulties may lower the younger child's score. On the other hand it is necessary to distinguish

between passive and active vocabulary in children. The child, like the adult, recognizes appropriately to many words that he does not use. The number of words that the child knows and uses will depend on the number of words he has been exposed to and therefore a number of socioeconomic factors. By the age of 10 the subjects studied by Templin (1957) had a mean estimated passive vocabulary of 20 000 words, with a standard deviation of about 10 000. On the basis of these data and our own experience, the same test we used for adults can be used also for children from the age of 4-5 years, when related to the norms for children. In younger children special tests must be used (Siegenthaler 1954, Sortini 1964).

Table IV Differences between monaural and binaural discrimination scores in filtered speech test

	Age group 4-5		Age group 6-7		Age group 8-9		Age group 10-11
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean
Left ear superior to right ear test	5.4**	5.9	4.3**	4.6	2.2	5.8	6.1
Binaural test superior to right ear test	5.0*	6.1	1.5	7.6	2.2	5.3	5.6*
Binaural test superior to left ear test	-0.4	-7.2	-2.4	7.9	0.0	7.8	-1.1

Differences were calculated separately for each individual case correlated to the test object concerned. Significance level  $p \leq 0.01$  = \*  $p \leq 0.001$  = \*\*.



once in discrimination  
in left and right ear

0-5%  
6-9%  
10-14%

Asymmetry in the monaural test at various ages

oup In the youngest group the discrimination scores were poor, about 40% lower than oldest group

cording to Boothroyd (1970), high pass has a greater effect on children's than low-pass filtering. In his opinion poorer performance of children must be due to the increased difficulty of the test bularly. However, this cannot be the only r since the drop in score depends on the of filtering.

ech audiometry sensitized by frequency tion is mainly used for the detection of al hearing disorders (Bocca et al., 1954, ker 1958, Jerger, 1960, Korsan Bengtson, Palva & Jokinen, 1975). In addition age ed degenerative changes have an effect on mination (Palva & Jokinen, 1970).

he binaural test result and the monaural

discrimination ability of the left ear were significantly better than the monaural test results for the right ear up to the age of 12-13 years. Anatomical development, notably myelination, is incomplete in the first years of life. Maturation of hearing abilities is a gradual process which reaches its maximum at the age of 12-13 years (Siegenthaler, 1969, Fior, 1972). Since the developmental stage in the normal-hearing child is the same in both ears, an asymmetric test result may indicate a functional asymmetry in the auditory system at those levels where asymmetry can appear physiologically. The results may be due to cerebral dominance in hearing (Penfield & Roberts, 1959, Milner et al., 1964, Shankweiler, 1966, Palva & Jokinen, 1970). In normal young adults the compensatory mechanism of the centrencephalic system may exclude the effect of cerebral dominance in the filtered speech test.

Matzker (1958) found positive binaural test results in 34% of the children under 14 years of age. The difference between his results and ours is probably caused by differences in test ing technique.

The fact that the binaural discrimination did not significantly differ from the discrimination of the better ear shows that the binaural synthesis of two different frequency bands is not affected in children.

## ZUSAMMENFASSUNG

140 Probanden im Alter von 4 bis 19 Jahren mit normalem Hörvermögen wurden Sprachtests mit normaler und mit filtrierter Sprache unterzogen. Alle Versuche wurden mit den von Erwachsenen benutzten Wörterbüchern durchgeführt. Nimmt man die Gruppe der 16-19-jährigen als Vergleichsmaßstab, so nahm die Fähigkeit normale Sprache zu verstehen bei den 4-9-jährigen mit dem Alter deutlich zu und bei filtrierter Sprache entsprechend in der Gruppe bis zu 11 Jahren. Diese Ergebnisse zeigen, dass Hordiskriminationstests schon im Kindesalter durchgeführt werden können und zwar—werden die Normen eingehalten—mit denselben Methoden wie bei Erwachsenen. Bei filtrierten Tests waren bis zu 12-13 Jahren hin die binauralen Ergebnisse und die monaurale Diskriminationsfähigkeit des linken Ohres erheblich besser als die monauralen Ergebnisse mit dem rechten Ohr.

S D	14-15		16-19	
	Mean	S D	Mean	S D
80	28	54	44	88
64	16	45	23	50
85	-12	59	-01	93

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## OTOTOXICITY OF TOPICALLY APPLIED GENTAMICIN USING A STATISTICAL ANALYSIS OF ELECTROPHYSIOLOGICAL MEASUREMENT

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Ototoxicity of topically applied gentamicin was in guinea pigs. 0.3% gentamicin was instilled in the middle ear cavity and Ringer's solution was in the other side and the difference in the microphonics measured with the round window mode was analysed statistically. Instillation of Ringer's solution in the middle ear cavity for 1 day did not cause any significant sensorineural hearing loss but on the 2nd day of instillation significantly reduced responses observed compared with the responses from non-instilled ears followed by partial recovery starting on the 3rd day.

When gentamicin 0.3% was instilled into the middle ear cavity significant deafness occurred 24 hours and highly significant deafness on the 2nd and 3rd day. The usage of gentamicin ear drops of the current formula should be discouraged until a better formula is developed.

of hearing impairment following gentamicin using an electrophysiological method were done by Lundquist & Wersall (1966), Soda et al (1968), Hawkins et al (1969), Brummett et al (1972) using guinea pigs. The drug was administered systemically in very high doses, viz 100 mg per kg for 7 days (Lundquist), 100 mg and 200 mg per kg for more than 19 days (Hawkins) and 50-100 mg for 4 weeks (Brummett).

The effects of topically applied gentamicin have been studied only by Wersall et al (1969), Webster et al (1970, 1971) and Stupp et al (1973) who have made histological studies. Since there is no experimental work on the effects of topically applied gentamicin on the ear audiogram this work was undertaken to find out if clinically prescribed gentamicin ear drops can cause deafness.

### METHOD AND PROCEDURE

A total of 85 guinea pigs of mixed sexes and of tortoise shell colour were employed in this study. Of these animals, 3 had middle ear infection, 2 had bilateral sensorineural deafness and 4 died of anaesthetic accidents. The guinea pigs were obtained from our central animal house where they have been interbred for the last 10 years. The body weight of the animals used was between 250 and 350 grams and all showed normal Preyer's reflex indicating detectable hearing.

Under local anaesthesia with 1% Xylocaine, an incision, 5 mm in length, was made at the retro-auricular region on the posterolateral part of the bulla. Two pin holes were made on the tympanic bulla, one for applying the test solution and the other for equilibrating the pressure in the middle ear cavity during the instillation of the test solution. The middle ear cavity was filled up with ear drops which were diluted with Ringer's through a pin hole using a 26 gauge needle, the middle ear cavity of the other side was filled with Ringer's as a control, and the animal was returned to the cage for a few days until required.

To measure the cochlear microphonic response, the animal was anaesthetised with 30 mg per body weight of Nembutal. A tracheostomy was performed and the animal was respiration supported with air using a small animal respirator. A muscle relaxant, Alloferin, was given from time to time, when necessary. The auditory bulla was exposed lengthen

Table 1 Cochlear microphonics from the control group (in dB scale) at 70 dB SPL

Frequency (Hz)	N	Mean (dB)	S D (dB)	For new observation	
				P=5%	P=1%
125	33	51.4	5.0	10.0	13.6
250	35	60.2	5.6	11.3	15.3
500	35	66.3	4.4	8.8	11.9
1 000	35	69.1	3.4	6.8	9.1
2 000	35	64.4	3.8	7.8	10.5
4 000	35	65.0	5.0	10.2	13.6
8 000	35	59.9	4.4	8.9	11.9
Average		4.5±0.8	9.1±1.5	12.3±2.1	

In the tables, 0 dB is equivalent to 0.1  $\mu$ V hence 60 dB is equivalent to 100  $\mu$ V

former incision, and a 3 mm hole was drilled in its posterior lateral surface. Using an operating microscope, the round window niche was located. The animal was positioned so that the round window was in a horizontal plane.

The test solution as well as all exudates was thoroughly removed using a small suction tube and a fine wick made of tissue paper. The corner of the middle ear cavity including the round window niche and tympanic membrane was carefully examined prior to the measurement of the responses. A silver wire electrode insulated except for the rounded tip, was placed on the round window membrane and an Ag-AgCl electrode was placed at the corner of the incision wound for the reference electrode.

The sound stimulus generated by an oscillator and loudspeaker was introduced into the external auditory canal through a tube and adaptor and the intensity of the sound was measured at the ear drum level using the same adaptor with a calibrated probe tube microphone. An octave band filter was used to obtain a better signal noise ratio.

Input-output function curves of the cochlear microphonics for seven frequencies viz 125, 250, 500, 1 000, 2 000, 4 000 and 8 000 Hz were made from all the animals except for those which showed no measurable response at 70 or 80 dB SPL. For these animals, the maximum response and the sound pressure

required for this response, were measured in order to distinguish sensorineural deafness from conductive deafness.

## RESULTS

### (1) Cochlear microphonic responses from non-treated guinea pigs

As a control, 35 guinea pigs were used in the study. The R M S voltages of cochlear microphonics under various frequencies and under various sound pressures were measured using a B & K microphone amplifier. The voltages were converted to a dB scale  $\alpha$  being set to 0.1  $\mu$ V r m s. Use of a dB scale facilitated easy comparison and analysis of the results, and the conversion of this dB change to the corresponding voltage can easily be made using a semi log chart.

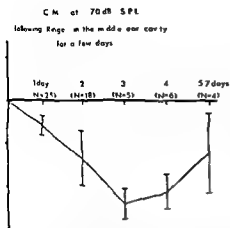
As shown in Table 1, the amplitude of the cochlear microphonic responses at a sound pressure level of 70 dB SPL was maximum at 1 kHz and this frequency gave the most stable output among the different animals while the maximum variations of the observed voltages were at 250 Hz.

The mean output for the 1 kHz at 70 dB SPL was 69.1 dB (re 0.1  $\mu$ V) which is equivalent to 280  $\mu$ V. Since its standard deviation (hereafter S D will be used) was 3.35 dB according to the Student's two-tailed *t* test, new observa-

Table 2 Cochlear microphonics at 70 dB SPL following Ringer instillation in the middle ear cavity for 24 hours

Frequency (Hz)	N	Mean (dB)	S D (dB)	For new observation	
				P=5%	P=1%
125	20	50.4	5.2	10.8	14.8
250	25	57.2	6.6	13.5	18.3
500	25	64.2	5.6	11.6	15.7
1 000	25	66.0	4.1	8.5	11.5
2 000	25	59.9	4.3	8.8	11.9
4 000	25	61.6	6.4	13.7	17.8
8 000	24	58.5	5.2	10.7	14.6
Average		5.3±0.9	11.0±1.9	14.9	

The measurements were carried out after the middle ear cavity was carefully cleaned out: see text.



Mean hearing loss in 7 frequencies are averaged and S.D.s are expressed with vertical bars on either side of the line. The responses from non treated ears are the control

of 6.8 dB smaller than the mean (62.3  $\mu$ V) and 9.1 dB smaller than the mean ( $\mu$ B=100  $\mu$ V) could be decided to be significant hearing loss of  $P=5\%$  and  $P=1\%$  respectively

Using the same procedure, the significance of the hearing loss at each frequency was determined statistically

#### Ochlear microphonic responses following instillation into middle ear cavity

Malian Ringer's solution (pH adjusted to 7.4) was used for instillation into the middle ear cavity as a control and as the solution to be tested

Microphonic responses 24 hours after instillation of Ringer are summarised in Table II. The responses from the non treated group (Table I) from this group (Table II) have essentially the same pattern. When the difference of responses in Tables I and II was compared at each frequency the Ringer treated group showed responses  $2.0 \pm 1.3$  dB lower than those of the non treated group, but the difference was statistically not significant

The question arises here as to what would happen to inner ear function if the Ringer was instilled in the middle ear cavity for a much longer period. Our study revealed that the Ringer

caused some sensorineural hearing loss and considerable recovery followed within a week. The loss of hearing for each frequency was averaged and its S.D. was calculated (Fig. 1)

The reduction of the responses reached its maximum on the 3rd day, and recovery occurred from the 4th day. The most marked reduction of the responses occurred at 4 kHz and the recovery of the responses at that frequency appeared to be slow

#### (3) Results with gentamicin otic solution, 0.3% in middle ear cavity

The following procedure was carried out

Firstly, using Table II, the animals which showed significantly lower responses from the control side at any frequencies were omitted from the present study. This procedure was necessary in order to exclude the influence of any preceding inner ear disease or of insufficient ventilation of the animal due to undetected respiratory obstruction which may have caused a lower response

Secondly, the differences between the left and right ears were analysed statistically using the S.D.'s of Table II and the two tailed  $t$  test. The procedure seemed to give us a more precise picture regarding the hearing loss caused by the test drugs contained in the Ringer because the absolute response as well as the change of the response due to Ringer varies considerably from animal to animal, hence the comparison of the absolute voltages from the ears which had the test solution with

Table III Deafness following gentamicin otic (0.3% Full strength) at each frequency

Animal	Duration (Day)	Degree of hearing loss						
		125	25	5	1	2	4	8 kHz
1	1D	-	±	-	-	±	-	±
2	1D	±	±	±	±	+	±	±
3	1D	±	±	±	±	+	+	+
4	1D	-	-	-	-	-	-	±
5	2D	++	++	+	++	++	±	++
6	3D	-	-	-	±	+	-	+
7	3D	++	++	++	++	++	++	++

±, 30%  $\geq P > 5\%$  +, 5%  $\geq P > 1\%$  ++, 1%  $\geq P$

the voltage from the non treated ears would not be valid

In this series of experiments, 0.3% otic solutions were used

As is shown in Table III, the test solution for one day caused significant loss of hearing in one out of four animals. Instillation for 2 days and 3 days caused severe deafness at all frequencies (Table III)

## DISCUSSION

It is difficult to extrapolate a non toxic level of topical gentamicin from the histological data such as of Wersall (1969, 1971) who used 50% gentamicin solution for 1 to 4 days and 0.3% solution for 7 to 13 days and of Webster et al (1970, 1971) who used 3%, 6% and 10% solution, because cochlear impairment had already existed before vestibular disturbances became noticeable

Since their histological changes were more marked in the cochlear component than in the vestibular component it would be reasonable to assume that cochlear impairment may have occurred well below the concentration of gentamicin they used topically

Stupp et al (1973) also demonstrated severe damage of the hair cells in all turns of the cochlea following 0.1 molar gentamicin in the middle ear cavity for 3 days. 0.1 molar gentamicin is equivalent to 5.4% solution. With our electrophysiological technique it is much easier to detect accurately the degree of hearing loss in terms of dB scale expressed as the decrease of the cochlear microphonics

Our results from guinea pigs indicate that the toxic level of topically applied gentamicin is less than 0.3% and is effective within 24 hours

There may be some difference in susceptibility to the drug from species to species. Judging from the above results guinea pigs appear to be approximately 10 times as vulnerable as cats. Similar results have been obtained following topical application of

chloramphenicol on the round window of cat (Patterson & Gulick, 1963) and guinea (Gulick & Patterson 1964). It appears however, that topically applied gentamicin is able to cause an ototoxic effect

### *Ototoxic level of the gentamicin*

It is generally agreed that a normal clinical dose of gentamicin in systemic use (3 mg/kg body weight) does not cause any ototoxic effect, provided the kidney is functioning normally

Study of Jackson & Arcieri (1971) shows that the ototoxic level of gentamicin in humans is above 12 µg/ml of plasma

Another study by Winters et al (1971) showed that the ototoxic level of gentamicin serum 12 µg/ml can be reached by injecting 4 mg/kg body weight

Chisholm et al (1973), injected gentamicin 80 mg intramuscularly into a dog of 26 kg body weight and the dog showed a peak serum level of approximately 10 µg/ml at 1 hour and a peak tissue fluid level of 6 µg/ml at 3 hours following the injection. His peak serum level is in good agreement with the result of Winters et al

No work has been published on the ototoxic level of gentamicin in the inner ear fluid. If we assume that the ototoxic level of the drug in the serum is the same as in the inner ear fluid and just ignore all of the facts about the dynamics of the inner ear fluids we can calculate how much gentamicin in the blood can be transferred to the inner ear through the round window membrane

From our results 0.3% otic solution for 1 day caused deafness in one out of four animals hence we regard this as the toxic level so far as the duration is concerned. Since 0.3% (3 mg/ml) is the level of toxic concentration in plasma (Jackson & Arcieri 1971) the inner ear fluids which have a volume of approximately 30 µl will need approximately 0.4 µg gentamicin to reach this toxic level and this means 0.13 µl of 0.3% gentamicin solution has to be diffused to the inner ear across the membrane. Only 0.4% of the total inner ear

has to be replaced by the otic solution in  
ulla for this level to be reached

## CONCLUSION

tical analysis of the cochlear responses  
carried out and quantitative analysis of  
earring loss was easily made in terms of dB  
ng loss as well as of probability. The  
ve hearing from the guinea pigs which  
ringer s in the middle ear cavity for more  
one day was found to decrease signifi-  
y but the reason for this could not be  
ined successfully. The decrease was re-  
ble.

r result indicated that even 0.3%  
mucin solution is toxic to the inner ear if  
ed into middle ear cavity for more than 24  
> There is a need to establish some  
ard technique and authorized labora-  
> to test the ototoxicity of all new and  
dy available otic solutions.

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## ZUSAMMENFASSUNG

Ototoxizität des Gentamicins tropfenweise in die  
eingeführt wurde an Meerschweinchen studiert.  
Gentamicine wurden in die eine Ringersche  
vkeit in die zweite Mittelohrenhöhle eingeführt und  
Unterschied des cochleären Mikrophonpotentials  
\*ten mit Hilfe von Elektroden an den Rundfenstern

\* 1. dritten Tag wurde die Schwerhörigkeit be-  
end. Vom vierten Tag der Behandlung mit der Ring-  
hen Flüssigkeit an erfolgte eine teilweise Genesung  
der Schwerhörigkeit. Als 0.3% Gentamicin in die  
elohrenhöhle eingeführt wurden, erfolgten eine

signifikante Schwerhörigkeit 24 Stunden später und eine  
sehr signifikante Taubheit am zweiten und dritten Tag.  
Der Gebrauch von Gentamicin-Ohrentropfen in der  
bisherigen Zusammensetzung sollte daher eingestellt  
werden, bis eine bessere Zusammensetzung gefunden  
wird.

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## CONGENITAL STAPES FIXATION, SYMPHALANGISM AND SYNDACTYLIA

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**Abstract** An autosomal dominant hereditary syndrome is described consisting of congenital deafness, characteristic finger and toe deformities with absence of the proximal interphalangeal joint together with syndactylia. The syndrome has been observed in 5 members of a Danish family, and the study confirms the autosomal dominant trait.

Symphalangism was first described by Mercier (1838) who reported a French family having only two phalanges on each finger. More and more reports have been published since then on symphalangism, partly as an isolated phenomenon, partly in combination with deafness, other hand and foot deformities and malformations of the lower arm. Cases have been registered in families throughout five continents, including China, Japan and Peru (Cushing, 1916; Strasburger, 1965).

The latter author has described the largest family with a total of 684 individuals, of whom 351 were affected. 'A considerable number' of these patients had congenital conductive hearing loss and operation revealed osseous fixation of the stapes.

Proximal symphalangism is termed in the Anglosaxon literature 'Talbot fingers' after John Talbot who was the first Earl of Shrewsbury and who fell during the battle of Castillon in 1453. The Earl plays a part in Shakespeare's King Henry VI, but no mention is made of his finger deformity. This diagnosis was con-

firmed at the time his grave was opened in 1874.

The combination of congenital stapes fixation and symphalangism was first described by Vesell (1960) and later by Gorlin (1970).

### MATERIAL

The material consists of a family of 21 individuals distributed over three generations. 5 had a combination of congenital hearing impairment, proximal symphalangism and syndactylia. 16 remaining members of the family are shown in (Fig. 1).

#### Case 1

The proband is now a 13 year-old girl who suffered from a hearing loss since her childhood and who has congenital symphalangism and syndactylia of the hands and feet. The patient's hearing loss brought about speech retardation and at the age of 4 years audiological treatment was commenced with good effect. Speech development was normal. Audiometry showed a conductive hearing loss on both sides with discrimination loss and it was impossible to obtain a stapedius reflex on either side.

At the age of 55 years the first left side explorative tympanotomy was performed during which a normal ear drum was found and there was no deformity of the bones of the ear.

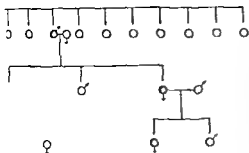


Fig. 1. Pedigree of the family

but osseous fixation of the footplate of the stapes was present. Indication for stapedectomy was not considered to be present at this age. The hearing remained unchanged throughout the following years. A stapedectomy was carried out at the age of 10 years during which it was found that the crus longum incudis was elongated and the incus deformed and fixed. Stapedectomy according to the method of House was then performed. A similar operation was done on the other ear 4 months later. During this procedure it was found that the crus longum incudis was deformed and that the footplate of the stapes was fixed. A satisfactory permanent improvement in hearing was obtained by both operations (Fig. 2).

The deformities of the hands consisted of syndactylia between the base of the 4th and 5th fingers together with ankylosis of the middle joints of the 4th and 5th fingers. Correspond-

ing symmetrical changes were present in the feet (Fig. 3).

X-ray examination revealed symmetrical changes with fusion of phalanx 1 and 2 of the 5th finger, which was also shorter than normal (Fig. 4). The joint between the corresponding phalanx of the 4th finger was incomplete, and the remaining fingers normal apart from the syndactylia. It was seen that in the wrist the capitate and hamate were fused, as were the trapezoid and trapezium. This was so in both wrists, in such a way that the distal part of the wrist in actual fact merely consisted of two bones. There was a corresponding fusion in the foot of the 1st and 2nd phalanx of the 3rd, 4th and 5th toes, together with fusion of the talus and calcaneum. There was in addition block formation of the cuboid and lateral cuneiform.

### Case II

A 39-year-old man, father to the proband, suffered from hearing loss from childhood. Otological examination revealed a narrow external meatus, but normal ear drums. The audiogram showed bilateral severe conductive hearing loss, and no reflexes could be obtained from the stapes on either side. In addition the patient had syndactylia between the 4th and 5th fingers together with ankylosis of the proximal interphalangeal joints of the 4th and 5th fingers on both hands. Similar changes were present in both feet.

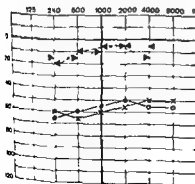
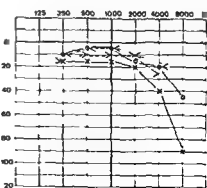
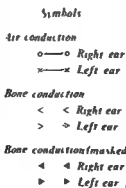
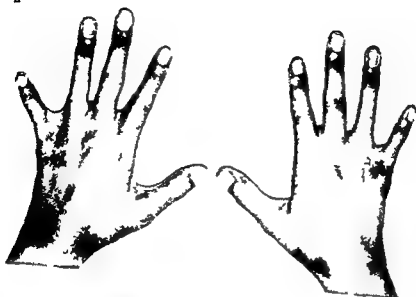


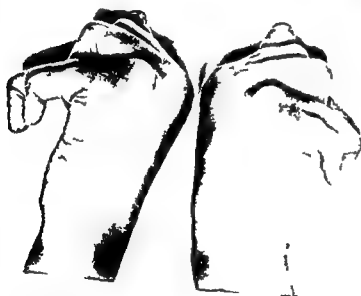
Fig. 2. Pre and postoperative audiograms



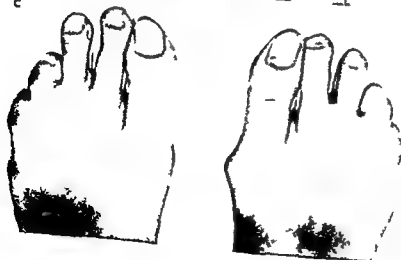
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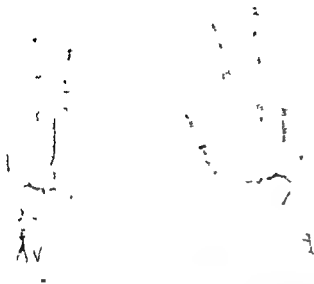
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*Fig 3 Typical deform and feet with syndactyly of the 4 ulnar digits (a-c). Talar-interphalangeal joint and 5th fingers present on the hands (b)*



*Fig 4* Roentgenogram of the hands of the patient (case I) showing fusion of phalanx 1 and 2 of the 5th finger and an incomplete joint between the corresponding phalanges of the 4th finger. Note fusion of both the capitate and lunate and the trapezoid and the trapezium

### III

12-year-old man, father to patient no. II, had deafness from poor hearing for many years. Audiogram showed bilateral conductive hearing loss. The ear drums were fibrous but movable. There were symmetrical finger and toe deformities with syndactyly between the 4th fingers and toes, but only the proximal phalanx of the 5th finger showed ankylosis.

### IV

12-year-old woman, sister to patient no. II, had a hearing loss of approximately 20–25 dB since childhood, particularly in the discant and especially in the left ear. Ear drums were fibrous but movable in Siegle's fundus. No stapes reflexes. The 4th ulnar fingers of both hands had syndactyly.

### V

5-year-old girl with congenital hearing loss, daughter of patient no. IV. The audiogram showed a conductive hearing loss of 40–50 dB. Radiography showed sclerosis of the oval window on the left side. The child had talipes equinus-varus at birth in addition to

syndactyly between the 1st and 2nd toe on both sides. Syndactyly was also present between the 4th ulnar fingers, as was ankylosis of the middle joints of the 3rd, 4th and 5th fingers of both hands.

## DISCUSSION

Reduced hearing in childhood occurs in Europe and North America with a prevalence of 0.3–0.03 (Brown, 1973; Fisch, 1973). Approximately 50% of these cases are hereditary, the 35% autosomal recessive, 10–15% autosomal dominant and approximately 2% X-linked recessive (Fraser, 1971; Nance, 1971). The combination described of congenital stapes fixation, proximal symphalangism and syndactyly thus belongs to the group of autosomal dominant hereditary diseases (Gorlin, 1970; Strasburger, 1965).

Histological studies of congenital stapes fixation (House, 1958; Steele, 1969) have shown a lack of development of the annular ligament and a bridge of cartilage, which becomes, at a later date, the site of ossification. Congenital stapes fixation can occur as an isolated development abnormality (Bernstein, 1966), but

most often it is seen in connection with other malformations such as in these cases with symphalangism, though it can also be seen together with severe deformities such as acrocephalo-syndactylia (Bergstrom & Neblett, 1972) Congenital stapes fixation in combination with other malformations of the hands and feet, but without an accompanying symphalangism has been described by Spoendlin (1974) and Schulthess (1974) In contrast to otosclerosis, the hearing loss with congenital stapes fixation does not progress and is of a purely conductive type, the audiogram showing a typical flat curve (House, 1958).

In order to obtain more information as to the nature of congenital hearing loss it is important that any accompanying malformations be registered It is also important that in cases where bone deformities are present a possible hearing loss should be borne in mind, in order that adequate treatment can be instituted as early as possible.

## ZUSAMMENFASSUNG

Es wird ein autosomales, dominantes erbliches Syndrom beschrieben, das aus angeborener Herabsetzung des Gehörs, charakteristischen Deformitäten der Finger und Zehen, Mangel an proximalen Interphalangealgliedern sowie Syndaktylie besteht Das Syndrom wurde bei 5 Mitgliedern einer dänischen Familie gefunden Durch Untersuchungen wird die autosomale Dominanz bestätigt

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## ACUTE OTITIS MEDIA

### *A Clinical, Bacteriological and Serological Study of Children with Frequent Episodes of Acute Otitis Media*

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A series of episodes of acute otitis media was studied with reference to bacterial findings and specific antibody responses in 48 children with histories of frequent episodes before. *D. pneumoniae* and *H. influenzae* were the most frequently isolated pathogens. Re-isolations were often made in episodes with slow or therapeutic failure. Most children harboured organisms in nasopharynx even when they had no signs of otory tract infections. Homologous titrations were only in few cases and never with pneumococcus type 1 only once with *H. influenzae* type 1. Specific responses were demonstrable generally in children over 2 years of age. *D. pneumoniae* type 3 and *H. influenzae* type b generally provoked antibody response. No levels indicating immunoglobulin deficiencies were found in the children.

Otitis media is a complication to an upper respiratory tract infection mostly confined to children under 10 years of age. In most cases the course is uncomplicated and the number of otitis episodes during their childhood is low. Many children, however, contract repeated infections, implying a risk of hearing impairment. The reason for repeated infections is obscure in most cases.

There have been suggestions that anatomical differences in the mastoid process or in the nasopharynx or disturbances in the immune function may be of importance (for review see Bluestone & Shurin 1972).

Some workers have pointed to the fact that during long periods of time many children

harbour in the nasopharynx the principal pathogenic bacteria found in acute otitis media and sinusitis, *Diplococcus pneumoniae*, *Haemophilus influenzae* and *Streptococcus pyogenes* ( $\beta$  streptococci), even though they show no signs of infection (Masters et al., 1958; Willard & Hansen 1959; Box et al., 1961; Turk & May, 1967). It has been suggested that a bacterial upper respiratory tract infection such as acute otitis media or sinusitis may depend on a viral infection which destroys the mucociliary barrier and so permits a secondary invasion by the pathogenic bacteria (Davidson 1972).

The possibility of an immature immune system with insufficient antibody formation against infecting bacteria has also been proposed by some authors as a reason for repeated middle ear infections in small children (Bjuggren & Tunevall, 1952; Sloyer et al., 1974).

In a study of the immunoglobulin levels in infancy and childhood in healthy Swedish children, Johansson & Berg (1967) showed that the IgG level remained low during the first years of life. At the age of 3-5 years, the level was about two-thirds of the adult level. The IgM level, on the other hand, was 70% of the adult level at one year of age, which level was usually reached at the age of four.

Studies concerning the prevalence of demonstrable specific antibodies against the main pathogenic bacteria isolated in acute otitis media have shown that the amount of such antibodies is low until the children are 3-4 years old as regards  $\beta$  streptococci and *H influenzae* (Bjuggren & Tunevall 1952, Hansson & Holm, 1961, Branfors Helander et al, 1973). Antibodies against *D pneumoniae* have been shown to appear somewhat earlier (Gundel & Schaefer, 1931, Bjuggren & Tunevall, 1952, Sloyer et al, 1974).

The present investigation is a study of a series of acute otitis media infections in a group of children who had suffered several previous episodes of otitis media. The study was performed in order

- 1 to reveal whether the pathogenic species isolated at the first studied acute otitis media infection could also be demonstrated at subsequent episodes. An additional aim was to investigate if the children of the present study harboured in the nasopharynx the main pathogens of the upper respiratory tract (*D pneumoniae*, *H influenzae* and  $\beta$  streptococci) at free intervals (no signs of infection)
- 2 to analyse the children's ability to react with a demonstrable immune specific response at otitis media infections by *D pneumoniae*, *H influenzae* and  $\beta$  streptococci. A study of the serum levels of IgG, IgA and IgM was also included
- 3 to investigate the possible influence of a serological response on the course of the infection and on the protection against homologous relapses

## MATERIAL AND METHODS

### Patients

The study comprised 48 children (27 boys and 21 girls) between 9 months and 12 years of age with clinical signs of acute otitis media. The study started in March 1973 and ended in August, 1974. The longest observation period in any child was 18 months and the

shortest one was 6 months. All of the children had had several episodes of acute otitis media previously most of them having had at least two episodes during the preceding 12 months.

In total the 48 children had 103 episodes during the investigation period. Unfortunately, 20 of the recurrences occurred at times when observation by us was not possible. Therefore, the study comprised 83 episodes of acute otitis media. The patients were followed after 14 days and the otitis considered healed when the tympanic membrane was normal and mobile and the child was without signs of upper respiratory tract infection. Audiology was taken in children with suspected hearing impairment.

All children except one were treated with oral antibiotics for 10-14 days. This had shown allergic manifestations on administration of different antibiotics and was therefore treated only with decongestants and antihistaminic drugs. Forty-one patients were treated with penicillin V 40-50 mg/kg and day in 4 equal portions. Three episodes of infection in this group of children were treated with ampicillin 50-70 mg/kg and day in three equal portions. In one of these latter infections therapy was changed from ampicillin to chloramphenicol because the patient harboured an ampicillin resistant strain of *H influenzae* type b. Six patients with history of exanthema in connection with penicillin therapy were given erythromycin stearate 40-60 mg/kg and day in four equal portions. All patients were also treated with nasal decongestants. No allergic manifestations were noted in any of the patients during the investigation. Samples for bacterial culture were taken from the nasopharynx (NFW) on day one in all 83 instances and on day 14 in 14 instances with the technique earlier described (Herberts et al 1971). Samples from the middle ear secretion were taken on day one in 46 instances. In addition samples from the NFW were taken from 38 of the 48 children at least 1 month after an acute otitis media infection.

## e) Bacterial findings

	D pn	H infl	$\beta$ str	Mixed path cult	Ord gr	Neg cult	Total
Sample	35	19	5	13*	11	-	83
Sample	10	4		2*	3	17	36

\* D pn + H infl | D pn +  $\beta$  str | H infl +  $\beta$  str | D pn +  $\beta$  str + St aur + Neiss  
 pn +  $\beta$  str + St aur + Neiss | H infl +  $\beta$  str

The child was without any signs of respiratory tract infection (these were denoted interval samples). Samples for serological analyses were obtained from 47 children with recurrent serum samples taken at 59 days of infection.

## Bacterial cultivation technique

Samples were incubated within 2 hours locally at 37°C on blood and gentian violet plates and in elevated CO<sub>2</sub> atmosphere on ematin agar plates. The cultivation and identification procedures used have been described previously (Branefors Helander et al 1973). The following bacterial species were tested as potentially pathogenic: *D. pneumoniae*, *H. influenzae*,  $\beta$  streptococci, *Staph. aureus*. In the ear samples, *Neisseria* (non specified) was also denoted as potentially pathogenic. The following bacteria labelled as indigenous growth: *Neisseria* (specified),  $\alpha$  streptococci, diphtheroids, *Staph. albus* and *Neisseria* (in the NF slides).

## Serological tests

**Agglutination determinations.** All serum samples were kept at -70°C until the tests were performed. The IgG, IgM and IgA concentrations were determined by the radial immunodiffusion method described by Mancini (1965) slightly modified (Nilsson 1968). Preparation of the agar plates: goat anti-human IgG, IgM and IgA sera (Hyland Laboratories) were utilized. A standard human serum for immunological determination of plasma proteins (Behringwerke AG) was used as reference and wells containing 100

75, 50 and 25% of the appropriately diluted standard serum were included for each plate. All serum samples were tested in duplicate.

**Pneumococcal anti-capsular antibodies.** were determined by the direct agglutination method. The sera (0.1 ml) were serially two fold diluted on flat bottomed Linbro trays and about  $3 \times 10^7$  pneumococci were added from a 6 hour old formalinized fluid culture. After incubation at 37°C for 2 hours and at room temperature for another 2 hours the tests were read with the aid of a magnifying glass. The initial serum dilution in the test was 1:2.

***H. influenzae* type b anti capsular antibodies.** were determined by means of the indirect haemagglutination method (IHA). Untreated human ORh-erythrocytes were coated with capsular antigen b. The material and the method have been described earlier (Branefors Helander 1973). The initial serum dilution in the test was 1:2.

***H. influenzae* O antigen antibodies.** were determined by means of complement fixation (CF) test in which a mixture of bacteria from 20 non-capsulated *H. influenzae* strains was used as antigen. The material and the method have been described earlier (Branefors 1973; Branefors et al 1973). The initial serum dilution in the test was 1:15.

**Antibodies against  $\beta$ -haemolytic streptococci (AST).** were determined by means of the anti streptolysin test (Winblad 1941).

## RESULTS

## Bacterial findings

The results of the bacteriological investigation are summarized in Table 1. The samples from



Table II The bacterial findings at the different episodes of acute otitis media

Pat	Age	Episode			Free interval
		I	II	III	
1 NJ	9 m	Pn <sup>3</sup>	ord gr <sup>a</sup>		$\beta$ str
2 LE <sup>2</sup>	11 m	Pn <sup>6</sup>	Hib	Pn <sup>23a</sup>	Pn <sup>23</sup> Hib
3 HH	17 m	Pn <sup>19</sup>	Hi <sup>a</sup>	Pn	Pn <sup>22</sup>
4 AE <sup>1</sup>	23 m	Pn <sup>19</sup>			ord gr
5 AL	23 m	Pn <sup>6</sup>			Pn
6 MM <sup>3</sup>	27 m	Pn <sup>19</sup>			-
7 LK <sup>2</sup>	27 m				no growth
8 CA	31 m	Pn <sup>3a</sup>	Pn <sup>23</sup>	$\beta$ str	Pn <sup>23</sup>
9 MO <sup>1</sup>	31 m	Pn <sup>3</sup>			Hi
10 AB <sup>1</sup>	31 m	Pn <sup>23</sup>	Pn <sup>23a</sup>		Pn <sup>23</sup>
11 PL	31 m	Pn	Pn <sup>23</sup> Hi	Pn <sup>19</sup> Hi <sup>a</sup>	ord gr
12 FJ	3 y	Pn <sup>6</sup>	Hi	Pn <sup>16</sup>	ord gr
13 HR	3.5 y	Pn <sup>19</sup>	Pn <sup>19a</sup>		Pn <sup>19</sup>
14 BJ <sup>1</sup>	4 y	Pn <sup>3</sup>			ord gr
15 AG	5 y	Pn <sup>23a</sup>	Pn <sup>6</sup>		Pn <sup>23</sup>
16 DK	5 y	Pn <sup>23a</sup>	Pn <sup>19</sup> Hi		Pn <sup>19</sup> Hi
17 OA <sup>1</sup>	6 y	Pn <sup>3</sup>			Pn <sup>19</sup> Hi
18 NL <sup>1</sup>	6.5 y	Pn <sup>3</sup> Pn <sup>10</sup>	Pn <sup>14</sup> Hi <sup>a</sup>		Pn <sup>23</sup> Hi
19 PA	7 y	Pn <sup>19</sup>			-
20 PP	8 y	Pn <sup>3</sup>	Pn <sup>3a</sup>		Pn $\beta$ str
21 TH	9 y	Pn <sup>3</sup> Pn <sup>3a</sup>			ord gr
22 TR <sup>1</sup>	14 m	Pn <sup>19</sup> Hi <sup>a</sup>	Pn <sup>19</sup>	Pn $\beta$ str	Pn <sup>19</sup>
23 JA	28 m	Pn <sup>3</sup> Hib			Hib
24 SF	29 m	Pn <sup>6</sup> Hi <sup>a</sup>	Pn <sup>6</sup> Hi		Pn <sup>6</sup> Hi
25 IS	5.5 y	Pn <sup>3</sup> Hi			-
26 MF	17 m	Hi	$\beta$ str	Hi <sup>a</sup>	Pn
27 PH	22 m	Hib	Hib <sup>a</sup>	Pn <sup>23</sup>	Hib Pn <sup>23</sup>
28 MW <sup>1</sup>	25 m	Hi			ord gr
29 KG	3 y	Hi			Hi $\beta$ str
30 ML	4 y	Hi	Pn <sup>19</sup> Hi	Pn <sup>19a</sup>	Pn <sup>3</sup>
31 MD	4 y	Hi			$\beta$ str
32 SP	4 y	Hi	Pn <sup>6</sup>		-
33 LL	5 y	Hib			Hib
34 AK	5 y	Hi	Hib <sup>a</sup>		Pn
35 JS	7.5 y	Hib			Hib
36 AL	10 y	Hi			-
37 KJ	12 y	Hi			-
38 EF	8.5 y	Hi $\beta$ str			Hi
39 MJ	4.5 y	$\beta$ str			Pn <sup>6</sup> Hi
40 CN	8 y	$\beta$ str			ord gr
41 ST	34 m	ord gr			Pn <sup>6</sup>
42 HA	4 y	ord gr	ord gr <sup>a</sup>	$\beta$ str	Pn <sup>14</sup>
43 SC	5.5 y	ord gr			-
44 AJ <sup>1</sup>	5.5 y	ord gr			-
45 RL	6 y	ord gr			Pn <sup>19</sup>
46 KB	7 y	ord gr			-
47 AW	10 y	ord gr			-
48 LJ	10 y	ord gr <sup>a</sup>	Pn <sup>3</sup> $\beta$ str St aur		$\beta$ str

2 LE had one more episode with Pn and one with Hi II  
PL one with ord gr and 26 MF one with Pn

<sup>1</sup> <sup>2</sup> Signifies the number of additional episodes

<sup>a</sup> Free interval sample after this episode

the NF yielded *D. pneumoniae* as the sole pathogen in 35 episodes (42%) and *H. influenzae* in 19 (23%) while  $\beta$  streptococci was found as the sole pathogen in only 5 cases. A

culture with two or more pathogenic species was found in 13 infections

In 36 cases samples were taken not only from the NF, but also from the ear secretion (Table I). Pathogenic bacteria were isolated from the ear secretion in 16 cases and in 1 of these the same species were found in the NF. In one child *H. influenzae* and  $\beta$ -streptococci were isolated from the ear and  $\beta$  streptococci from the NF. The culture from the ear secretion was negative or yielded only non pathogenic species in 20 cases. 19 of them yielded pathogens in the NF. No growth in the NF was in these cases as well as in most others recorded as moderate or heavy. It was also noted that in the NF the pathogenic bacteria were often found as the sole species. All bacteriological results given in the following text are based on isolations from the NF.

Table II presents the bacterial findings in the first studied and the two subsequent infections for each of the 48 children. As regards pneumococci, the most commonly isolated serological types were 3 and 19 (9 and 13 isolates each), followed by 6 and 23 (7 isolates each). The seven other types found were represented by only one or two isolates each. (The serological typing of *Diplococcus pneumoniae* has been performed at Stat Serum Institut Copenhagen Denmark by E. Lund, M.D. to whom our thanks are due). Of the 30 isolations of *H. influenzae* were capsular type b, while the remainder were capsulated. Beta streptococci were often isolated in a mixed pathogen culture. It is noted that *Staph. aureus* was found in only 4 single infections. In this case (48 LJ) *S. aureus* was isolated together with  $\beta$  streptococci, pneumococci and *Neisseria* (non specified) from both the NF and the ear secretion. This patient had had a tympanic membrane perforation for about a week when the samples were taken.

As may be seen from Table II the bacterial species or sero-type that was isolated at the infection first studied was in most cases not observed at a subsequent infection.

Table III Immunoglobulin levels in mg per 100 ml serum in 47 children with frequent acute otitis media infections

No	IgG			IgA			IgM		
	Mean	S D	Range	Mean	S D	Range	Mean	S D	Range
7	800*	374	543-1 559	49 4*	29 8	18-106	58 1	33 2	23-111
11	781	257	469-1 250	109 5*	51 9	29-200	91 2*	34 3	59-176
11	1 078*	361	640-1 854	106 0*	71 3	18-270	84 7	47 0	25-157
18	1 186*	385	614-1 930	129 6*	49 7	80-278	83 5	41 0	24-168

Mean values indicated with \* are significantly higher ( $P < 0.05$ ) than corresponding values given for healthy Swedish children by Johansson & Berg (1967)

ance, there had often been a change from pneumococcal type to another or from pneumococci to *H. influenzae* or vice versa. In the children under 3 years of age pneumococci were found nearly twice as often as *H. influenzae* while they were about equally common in the older children. Cultivations of ordinary growth were, with few exceptions, found in children over 3 years of age.

After treatment, *D. pneumoniae* was re-isolated from 14 of the 47 infections where it had been initially isolated. In spite of its initial frequency, type 3 was never re-isolated, either as it was found in a subsequent infection or in the interval. In any child *H. influenzae* was isolated after treatment in 23 out of 30 cases. In five of these the strains were of capsular type b. In addition, *H. influenzae* was isolated in moderate or heavy growth after therapy in 14 cases where it had not been isolated initially.

At a free interval, samples from the nasopharynx were obtained from 38 children and in 29 of these no pathogenic bacteria could be demonstrated (Table II). *D. pneumoniae* was the pathogen most often found (21 patients), followed by *H. influenzae* (13 children). As regards pneumococci, the same sero-types as in the previous infection were found in only 4 children (2 LE, 10 AB, 15 AG and 24 SF). It was also observed that 3 children (8 CA, 16 BK and 27 PN) shortly afterwards had new episodes with the same sero-types isolated as in the free interval. It is noteworthy that no less than 5 of the 6 children with *H. influenzae*

b infections had this micro-organism in a free interval sample taken 2-6 months later. It is also notable that as many as 5 children were found to harbour  $\beta$ -streptococci at a free interval. One of these children (48 LJ) had a new episode of acute otitis media within one month, at which infection  $\beta$ -streptococci could be isolated.

#### Serological results

**Immunoglobulin determinations.** In Table III the results of the immunoglobulin determinations are summarized. It may be noted that the measured levels displayed a wide range for all age groups in both IgG, IgA and IgM. However, in subsequent samples from individual children, very consistent values were obtained. In none of the children in this study was general hypogammaglobulinaemia observed. No IgG levels were below the normal range and only a few children had values for IgA or IgM that were lower than reported normal for uninfected children. On the contrary, many individual values and also most mean values were found to be significantly higher ( $p < 0.05$ ) than those reported by Johansson & Berg (1967) for healthy Swedish children.

**Pneumococcal anti-capsular antibodies** (agglutination test). These antibodies were determined in consecutive serum samples by means of homologous pneumococci from 22 children with isolation of this species. The results summarized in Table IV show that more than half the number of children had demon-

Table IV Agglutinins against homologous serological types of *D. pneumoniae* in consecutive serum samples from 22 children

	Demonstrable titres	
	initially	7-14 days later
Number of samples with antibodies	13/22	17/22
Titre range	1:2-1:16	1:4-1:16

strable titres already in the initial samples. Five of these children were under 3 years of age. In the sample taken 7-14 days later only 4 more children showed a titre. Antibodies against type 3 were found in 6 out of 7 children tested, a titre increase (>twofold) being observed in 4 of them (1 NJ, 18 NL, 23 JA and 48 LJ). In only 2 other children, infected with types 23 and 6, was a titre increase observed (10 AB and 24 SF). The brothers 13 HR and 22 TR were the only children with demonstrable initial antibodies against type 19.

*H. influenzae* type b anti-capsular antibodies (IHA-test). This test was performed in serum samples from 47 children and consecutive samples were investigated from the 6 children with isolation of *H. influenzae* type b. The results are summarized in Table V. Eight of the 41 children (20%) with no isolation of *H. influenzae* type b showed demonstrable anticapsular-b antibodies. One of these children was under 3 years of age.

In 2 of the 6 children with isolation of *H. influenzae* type b, antibodies could be demonstrated even initially. In the second sample 2 more children showed demonstrable antibodies. It seems notable that 2 of the 3 children under 3 years of age showed increasing titres (2 LE and 23 JA). The third child under 3 years of age was 27 PH in whom no titre was revealed in any serum sample, despite repeated episodes and a carrier state of altogether 4-5 months duration.

*H. influenzae* O antigen antibodies. These were determined in consecutive serum samples from the 47 children by means of the

CF-test. The results are summarized in Fig. 1. a presents the results obtained from episodes with initial isolations of *H. influenzae*. It is seen that 10 children displayed titre increase (>twofold). The children whose sera revealed a titre increase or an initial titre of over 1:30 were all over 2 and most of them over 3 years of age. The 3 children without demonstrable titre were under 2 years of age (LE, 22 TR and 27 PH). The child with exceptionally large titre increase was 24, who also showed a titre increase against isolated pneumococcus type 19. In Fig. 1b titres demonstrated in the episodes with initial isolations of *H. influenzae* are presented. In 10 of these episodes *H. influenzae* was isolated after therapy but this did seem to affect the CF-titres. The mean age of the children without demonstrable initial titre was 43 months (9-72 months) as compared with 74 months (31-120) for those who had titres. This difference is statistically significant ( $p < 0.05$ ).

Anti-streptolysin titres (AST). In Table 6 the AS titres for the 47 children are summarized. Serum samples were obtained from 10 of the eight episodes with isolations of  $\beta$  streptococci. The results showed that 6 children under 2 years of age (22 TR and 40 MF) did not get any increasing titres while the other three (38 EF, 39 MJ and 40 CN) all 4 years of age, showed increasing titres (from 1:100 to 1:400 or 1:800). In the group of children from whom no  $\beta$  streptococci were isolated only half the number had a demonstrable AST ( $\geq 50$ ). Only 2 children both 4 years of age had a titre as high as 1:400.

Table V Agglutinin titres against capsular antigen b of *H. influenzae* tested with indirect haemagglutination method in 47 children

Isolation	Demonstrable antibodies	
	initially	7-14 days later
<i>H. infl.</i> type b	2/6 (1:8-1:32)	4/6 (1:8-1:32)
No <i>H. infl.</i> type b	8/41	

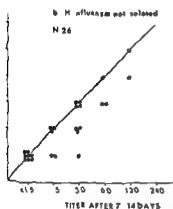
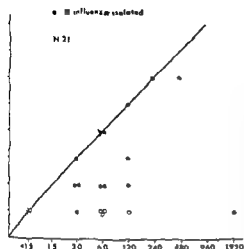


Fig. 1. CF titres in infections with (a) and without (b) isolation of *H influenzae*. Filled symbols indicate healing and open symbols therapeutic failure or slow healing.

### Results

Of the 83 episodes studied were considered healed at the control after 14 days. Sixteen episodes were not completely healed after 14 days but prolonged antibiotic therapy was not considered necessary. Therapeutic failure was noted in 2 children with two episodes each (22 TR and 27 PH).

The child 27 PH had two episodes at which *H influenzae* type b was isolated both initially and after therapy. No anticapsular b or CF titres were observed in any of the serum samples tested. The child 22 TR, who was not treated with antibiotics due to allergic manifesta-

tations had two long lasting infections with pneumococcus 19. He had an initial titre (1/8) against pneumococcus 19 even at his first infection but no titre increase could be demonstrated.

In the 13 infections with slow healing, pneumococci and *H influenzae* were isolated equally often (in four of these episodes isolated together). Of the slowly healing pneumococcal otitis episodes, only one was by type 3, in a child aged only 9 months. It may be noted that two of the seven slowly healing *H influenzae* infections were of capsular type b. In only one of the 13 children with slowly healing episodes was a specific antibody titre demonstrable initially. Six of the children, however, showed demonstrable titres in the samples taken after treatment and among those were the 2 children with *H influenzae* type b infections. None of them got a new episode of otitis media with this microorganism but 2 LE had three more infections with other bacterial species isolated. The children 10 AB and 24 SF had demonstrable titre increases against pneumococci but despite this immunological response, their infections healed slowly. The same pneumococcal types were also found after therapy and both children soon got new episodes with isolation of the same sero-types. Even at the free interval samples taken 2 and 5 months later, identical pneumococcal sero types could be isolated from the NF.

The children with episodes of acute otitis media which were considered healed after 14 days were mostly older than those with therapeutic failure or a slowly healing infection. Concerning the bacterial findings in this group of children, it may be noted that all episodes with  $\beta$  streptococci or indigenous

Table VI. Antistreptolysin titres (units/ml) measured in 47 children

Isolation	<50	50	100	200	400	800
$\beta$ -streptococci	1	1			2	1
No $\beta$ -streptococci	22	9	4	5	2	

growth were healed within 14 days, as were all but one episode with pneumococcus type 3.

As regards the serological reactions, it may be noted that specific antibodies were generally observed in the children with pneumococcus type 3. In children with well healing *H. influenzae* infections CF titres were mostly demonstrable in the initial serum sample in contrast to those with therapeutic failure or slowly healing infections.

It is also notable that in most of the 17 infections which did not heal properly, at least one of the initially isolated pathogens could be re-isolated after treatment, while re-isolations were more infrequent among the well healing infections (12/17 as compared with 27/66). This difference is not significant ( $0.05 < p < 0.1$ ).

Thirty-five recurrences were studied in 21 children. The mean age for the children who had one more episode during the observation time was 42 months and it was 29 months for those who had more than one. Seven recurrences occurred within 14 days after termination of the antibiotic treatment, and in four of them the same species were found at both infections (10 AB, 13 HR, 27 PH and 42 HA). The children 10 AB and 13 HR had episodes with isolation of pneumococci (Table II). From both children the pathogens had been isolated even after therapy at the previous infection studied. Both these children had had demonstrable specific antibodies and 10 AB even a titre increase. The child 27 PH had isolation of *H. influenzae* type b without any demonstrable anticapsular b titres while the fourth child had an episode of acute otitis media with isolation of *Neisseria* as sole species. Generally however bacteria of the same species were not isolated from the children at the first studied and at subsequent episodes of acute otitis media.

In only a few children could an epidemiological pattern be traced. The brothers 13 HR and 22 TR had altogether four episodes with isolation of *D. pneumoniae* type 19 starting in the older boy and proceeding in the family for 3-4

months. The children 1 NJ, 14 BJ and 41 were also siblings displaying an epidemiological pattern which was possible to follow about 4 months of infections with 1 pneumococci and  $\beta$  streptococci.

There was also a connection between children 2 LE and 27 PH who attended same day nursery both having infections with *H. influenzae* type b. The girl 2 LE had infection by this microorganism 4 months before 27 PH was included in the study. However, he had had three long lasting episodes with isolations of *H. influenzae* serotyped, during these months. The *H. influenzae* type b strain isolated from the N, as well as from the ear secretion of 27 PH proved to be resistant to ampicillin. Therefore this child was later treated with oral chloramphenicol with a good result. However, ampicillin resistant *H. influenzae* type b was still isolated from his NF 3 months later. Because of this a bacteriological examination of the children and the staff at the nursery made *H. influenzae* type b was isolated from 4 of the 10 persons examined but all 10 strains including that of 2 LE turned out normally sensitive to both penicillin and ampicillin.

Eleven of the 48 children had undergone adenoidectomy before the investigation started. All of them had had new episodes afterwards and in only 4 cases did the parents report that the operation had reduced the frequency of acute middle ear infections. Another 8 patients were operated upon during the last 6 months of the investigation period. In spite of this treatment all except one of them got new episodes of acute otitis media.

## DISCUSSION

In the present study of children with histories of frequent middle ear infections the results of the bacterial cultivations are in accordance with other studies carried out in recent years concerning acute otitis media with *D. pneumo-*

*influenzae* as dominant pathogens & Rundcrantz, 1967, Howie et al., 1972).

It has been proposed that one of the many reasons for frequent attacks of acute otitis media is a chronic adenoiditis (Davidson, 1967). This suggestion was based on the fact that several excised adenoids from children with frequent infections showed growth of the pathogenic bacteria generally isolated in this disease. However, some workers (Box et al., 1961, Turk & May, 1967) have found that children without respiratory tract infections harbour pneumococci or *influenzae* nearly as often as children with such infections. In contrast to such results Wil-Hansen (1959), Norstedt (1967), and Davidson et al. (1972) found the main pathogenic bacteria considerably less often in children than in those with an acute infection.

In the present study was found that most of the children harboured one or more of the main pathogens in their nasopharynx (NF) even during their free intervals. They showed no signs of infections. This implies that children with frequent attacks of acute otitis media are more prone than other children to harbour potentially pathogenic bacteria in the NF.

When the local resistance is lowered, e.g. by a preceding infection, these pathogens may engage the mucous membranes and give rise to upper respiratory tract symptoms.

In the present study it was noted that some children who harboured pathogens during a free interval shortly afterwards got an infection in which the same type of pathogen was isolated. This sequence was observed for infections by various pneumococcal types, except for type 3 which was one of the most frequently found types in the present investigation.

It should be mentioned that among the otitis media episodes only those caused by pneumococci provoked a demonstrable antibody response. In most cases Carlens (1943), too, was unable to report antibody responses against this pneumococcal serotype in children over one

year of age in his studies on acute otitis media. It may also be noted that all episodes of acute otitis media with type 3 were considered healed after 14 days, except one in a child aged 9 months. In none of these children was pneumococcus type 3 found after therapy. The observed good therapeutic results as well as the absence of recurrences at free intervals might be attributed to good immunogenic properties of this pneumococcal serotype.

The therapeutical results at the seven episodes of acute otitis media with *H. influenzae* type b isolations varied, with good healing in three, slow healing in two and therapeutic failure in two incidents. It is interesting to note that in all but one of these patients *H. influenzae* type b was found even after therapy. In all children with re-isolation after therapy a carrier-state was established as indicated by *H. influenzae* type b isolations in samples from NF taken 2-6 months later. Specific antibodies as tested by the IHA-method were demonstrable in sera of 4 patients who, despite a long term carrier-state, did not get a new episode with *H. influenzae* type b. One child in whom no serological response was observed had a second episode with *H. influenzae* type b and therapeutical failure was noted at both instances. In addition a carrier state for at least 3 months was seen.

The explanation for most re-isolations of *H. influenzae* type b is most probably the comparatively low sensitivity of this bacterial species against V-penicillin. The absence of recurrences despite a long term carrier state of *H. influenzae* type b might be explained by the fact that in these patients a specific antibody response was present.

Our observation that it is the small children who are most prone to get repeated attacks of otitis media is in agreement with the results of many other investigations (e.g. Bjuggren & Tunevall, 1952, Lundgren, 1972). However, homologous relapses were seen in only a few cases and these episodes mostly occurred within 30 days after the onset of the previous infection. These findings are in agreement

with those reported by Kamme et al (1970) for pneumococcal recurrences

The findings in this study that demonstrable specific antibodies against the three main species (*D. pneumoniae*, *H. influenzae* and  $\beta$ -streptococci) were mostly observed in children over 2 years of age is well in keeping with results reported by Bjuggren & Tunevall (1952) and, as regards pneumococcal episodes of acute otitis media, also with those reported by Sloyer et al (1974). The absence of a demonstrable, specific, humoral immune response in small children with acute otitis media might be the reason why they are more susceptible to subsequent attacks of otitis media with the same sero-type or species.

It has been postulated that one of the possible reasons for susceptibility to repeated middle ear infections is an immune deficiency (Davidson, 1966; Kiviranta, 1967). Kiviranta found that the average gammaglobulin levels in a group of 173 children with frequent attacks of otitis media were less than two thirds of so-called normal values. On the other hand Berg et al (1971), who studied American Indian children with chronic otitis media could not find any levels in the examined children indicating immunoglobulin deficiencies. Like the latter workers, we could not find any quantitative defects in the immunoglobulin levels in the children examined in the present study. On the contrary, many children showed elevated levels as compared with healthy children of the same age (Johansson & Berg, 1967). These higher values may be explained as caused by the repeated episodes with acute otitis media which was the criterion for selecting the children of this study.

## ZUSAMMENFASSUNG

Bei 48 Kindern die eine Anamnese von häufigen Otitis media Infektionen hatten wurde eine Folge von Erkrankungen mit besonderer Berücksichtigung von bakteriellen Befunden und spezifischen serologischen Reaktionen untersucht. *Diplococcus pneumoniae* und *Haemophilus influenzae* waren die am häufigsten isolierten pathogenetischen Keime. Bei wiederholter Untersuchung konnten oft erneut pathogenetischen Keime

isoliert werden und zwar bei Erkrankungen in gleicher Heilung oder bei Therapienversagen. Die 5 Kinder hatten pathogene Keime im Nasopharynx wenn sie keine Symptome einer Luftwegsinfektion wiesen. Rückfallerkrankung mit bakteriologischem Fund wie bei der Erstinfektion wurde nur in 1 Fall beobachtet, nie jedoch bei Infektion mit *P. coccus* Typ 3 und nur einmal bei *H. influenzae*. Spezifische serologische Reaktionen ließen sich hauptsächlich bei Kindern über 2 Jahren *Diplococcus* Typ 3 und *H. influenzae* Typ 1 finden. In den meisten Fällen eine immunologische Reaktion bei der Untersuchung der Immunglobuline fanden keinen Fall Konzentrationen die auf eine mangelnde Immunantwort andeuten.

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## AN EXTENDED APPROACH THROUGH THE MIDDLE CRANIAL FOSSA TO THE INTERNAL AUDITORY MEATUS AND THE CEREBELLO-PONTINE ANGLE

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**Abstract** The submeningeal approach through the middle cranial fossa to the internal auditory meatus and cerebello-pontine angle after cutting the tentorium cerebelli is described. It is in some way similar to that used by Morrison & King. The approach is achieved by drilling the petrous bone to the level of the compact plate of the sigmoid sinus sulcus and to the lumen of the lateral semicircular canal. In this way it is possible to visualise safely the entire auditory facial fasciculus, the region of the jugular foramen, the IX, X, XI, V and VI nerves as well as the lateral surface of the brain stem. In our opinion this way should be and is preferable to the translabrynthine approach through the mastoid process.

We describe an approach to the internal auditory meatus and the region of the cerebello-pontine angle beneath the meninges of the middle cranial fossa, after having drilled the upper part of the pyramid and after cutting the tentorium cerebelli.

A submeningeal approach to the surface of the pyramid through the middle cranial fossa has often been used in the past, particularly for drainage of this area in the case of infection, and also in head injuries. However, not until 1904 when R. H. Perry described the section of the VIII nerve in Meniere's disease, were various specific techniques involving this region developed, among which is the operative exposure of the internal acoustic meatus and the posterior cranial fossa by an approach through the middle cranial fossa.

The most extensive advocacy and the most

accurate description of these operations is from William House, who in 1961 demonstrated a technique of cutting part of the VIII nerve without damaging the function of the inner ear. Describing other techniques, House specified the exact indications for determining the approach to the meatus and the cerebello-pontine angle in the cases of a neuroma of the VIII nerve according to localization of the tumour. According to this classification a tumour with a diameter of over 8 mm, or that extending beyond the area of the internal acoustic meatus, should be removed by an approach through the labyrinth. Function of the inner ear has a relative value in determining the method of approach for removing the tumour mass. Thus the most important method which permits the widest approach to the internal acoustic meatus must allow for the following factors: tumour size and location, continuity of the facial nerve and control of bleeding in case of damage to larger blood vessels. An extended approach after section of the tentorium cerebelli, which was first suggested by Shulitz et al. in 1896 is not sufficient to view the region of the cerebello-pontine angle adequately. In our opinion, the approach *ad meatus* of House is also insufficient, especially in case of removal of tumours in the region of the bulb of the jugular vein, as those are localized medially in relation to the auditory

**fasciculus** According to the description Morrison & King (1973), Henderson uses each through the pyramid when re- ing tumours of the posterior cranial fossa, ough details of this method are unclear approach is similar to the combined ap ch used by Morrison & King (1973) but the translabyrinthine part of the on We usually open the labyrinth the middle fossa having the possibility the approach in the direction of the id sinus

### OPERATIVE TECHNIQUE

general anaesthesia, a 10 cm incision is through all the tissues in the preauricular just as in the approach through the mid cranial fossa. The entire surface of the amous temporal bone is uncovered and if ambdoid suture is not readily seen in the of the mastoid angle an additional incision ade in the post auricular groove, beginning the preceding incision and running in the on of the mastoid process. Using a cut burr a plate of bone approximately 5.5x3 is next removed.

After exposing the meninges a 20% solution Mannitol is given i.v. approximately 1.0/kg by weight. A fall in intra cranial pressure is after giving Mannitol and this allows separate of the meninges from the bone and to raise the temporal lobe in order expose the petrous part of the temporal . The dura mater is separated from the Enor surface of the pyramid to the level of sulcus of the superior petrosal sinus from ove and the hiatus of the facial canal from front. The procedure here is for decom- sion of the VII nerve according to Pulec ) or in exposing the internal auditory s for section of part of the VIII nerve ording to Fisch (1970). Next the superior osal sinus is gently dissected away from its dcus beginning from its orifice (to the enoidal sinus) and progressing in the direc on of the pyramidal apex.

Drilling the petrous bone is be

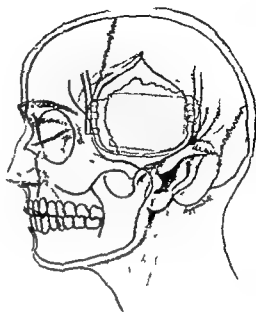


Fig 1

opening the mastoid antrum to the level of th compact plate of the sigmoidal sinus sulcu unveiling the lumen of the lateral semi-circle canal. Its course is a very important landmar in the successive steps of the operation. After opening the vestibule, the fundus may be reaclly located and the internal auditory meatu which is not opened until a sufficient amount bone has been removed for good visualizati of the tumour. The removal of bone is begi laterally in relation to the internal audito canal and progresses towards it. Such mea- ures ensure greater safety with regard to the VII nerve due to the fact that even in openi the internal auditory canal the vestibular pa- of the VIII nerve are located in the extre- lateral side in the fasciculus.

After a sufficient amount of bone has be- removed the dura mater (tentorium cerebel- is gently incised parallel to the upper border the pyramid. Next both flaps of meninges a- separated and supported by suture thread

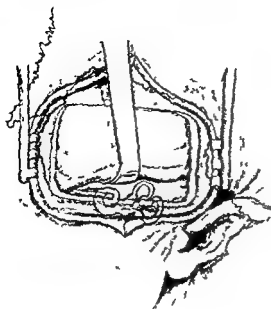


Fig 2

Such an approach gives us an exact view of the auditory-facial fasciculus, located in the field of operation. Inferiorly and somewhat laterally, the sigmoid sinus is seen together with the IX, X and XI nerves, while supero medially and from the top the V, VI and sometimes the III nerves. Somewhat deeper in relation to these structures is the cerebellum and the posterior inferior cerebellar artery. With further removal of pyramidal bone in the direction of its apex it

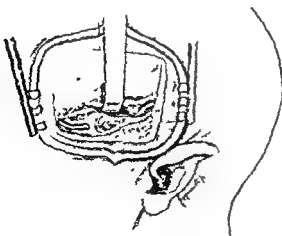


Fig 3



Fig 4

is possible to visualize the basilar artery and brain stem.

When the access to the tumour is sufficiently large and all the anatomical structures in the internal auditory canal and the area of the ear have been identified, we may then proceed to remove the tumour. The decision whether to remove the mass in fragments or in its entirety depends on its size and vascularity. Quite often it is possible to remove the tumour totally with its capsule from the surrounding tissue fairly readily. Blood vessels entering the capsule or those already damaged earlier are coagulated by the use of a bipolar electrode. The separation of a large tumour is usually impossible, so that fragmentary removal is necessary, and in this way the access to the tumour is improved. After removal of the tumour mass and complete haemostasis it is necessary to check the integrity of all the nerves, particularly the facial nerve. The next step is to suture the edges of the incised meninges (tentorium cerebelli). Temporal fascia is placed on the opened surface of the space of the middle ear and petrous bone. By this means additional separation of the middle ear from the cranial cavity is achieved which in turn guards against the liquorrhea and the possible spread of infection from the middle ear to the meninges. A delay of 15–20 min is necessary.

to allow for the dilatation of the temporal  
The removed plate of temporal bone is  
placed in its former place and is sutured with

A rubber drain is placed into the  
space for about 24 hours. Soft tissues  
are sutured in layers. In the postoperative  
period (2-3 days) Mannitol is given 1 v 1 g/kg  
weight. All patients receive antibiotics  
prophylactically.

## DISCUSSION

The removal of tumours of the VIII nerve  
extending beyond the internal acoustic meatus  
for tumours of the cerebello-pontine angle  
performed through a different approach from  
which have been previously described.  
This modification creates a new and improved  
possibility for surgical intervention in the region  
of the cerebello pontine angle. Thus the  
removal of the upper part of the pyramid up to  
the level of the bony plate of the sigmoid sinus  
laterally and to the lumen of the horizontal  
semicircular canal medially, enables the visualization  
of the entire auditory facial  
nerve, the bulbous of the jugular vein, the  
X, XI, V, VI and III nerves. In this way it is  
possible to visualize the anterior surface of the  
clivus and the lateral recess of the fourth  
ventricle as well as the lateral surface of the  
brain stem. The anterior inferior cerebellar  
artery is also well seen along its entire length  
from the basilar artery to its loop in the area of  
the orifice of the internal acoustic meatus.  
The approach described was used excep-  
tionally only in the situation when, according to  
Jesse (1964) the approach through the middle  
fossa was found to be insufficient during  
the course of the operation. This was the case  
when the tumour was found to be larger than  
anticipated and extended beyond the internal  
acoustic meatus. It is considered that this  
should always be the routine approach when  
the tumour is over 8 mm of diameter and de-  
mands a wider approach. This approach to the  
internal acoustic meatus and the anterior part  
of the posterior cranial fossa is considered to be

better than that passing through the mastoid  
process and the labyrinth, for the following  
reasons:

1. It gives a better orientation in the field of  
the operation,
2. it can be extended at any given moment,
3. it creates wider visualization of the area of  
the cerebello pontine angle, which allows  
freer manoeuvring to achieve haemo-  
stasis and especially in haemorrhage from  
the posterior inferior cerebellar artery or  
its branches,
4. removal of part of the temporal bone can be  
used as the first step (decompression) in  
the case of large tumours in the posterior  
cranial fossa,
5. it creates the possibility of visualization of  
all anatomical structures in this region from  
a better angle,
6. it enables a wider and thus a better  
approach to the internal acoustic meatus  
and the auditory facial fasciculus along its  
entire length, which is important in pre-  
serving the continuity of the VII nerve,
7. it creates facilities for decompression of  
the VII nerve,
8. communication with the middle ear is the  
same as in the approach through the  
labyrinth but with this technique it is  
easier to separate these two spaces,  
namely the cranial fossa and the middle  
ear, by placing temporalis fascia or a frag-  
ment of fascia lata on the surface of the  
pyramid,
9. it is well tolerated by patients,
10. in the case of complications it is easy to  
achieve access to the operated region.

## RÉSUMÉ

Les auteurs décrivent la voie submeningeale de la fosse  
cérébrale moyenne pour aborder le conduit auditif interne  
et l'angle ponto-cérébelleux après l'incision de la tente  
du cervelet. La voie ressemble un peu à celle utilisée  
par Morrison et King. On procède à fraiser le rocher  
jusqu'au niveau de la lame compacte de la gouttière  
sigmoïde et jusqu'à la lumière du canal semi-circulaire  
externe. On obtient ainsi en toute sécurité une vue

suffisamment large sur le fascicule acoustico facial, la région du trou déchiré postérieur, les nerfs IX, X, XI, V, VI et la partie latérale du tronc cérébral. Les auteurs trouvent cette voie préférable à la voie trans labyrinthique à travers la mastoïde.

## ZUSAMMENFASSUNG

Der submeningeale Zugang durch die mittlere Schädelgrube zum inneren Gehörgang und zum Kleinhirnbrückenwinkel nach der Inzision des Tentorium cerebelli wird beschrieben. Dieser Zugang entspricht in gewisser Weise dem, der von Morrison und King benutzt wird. Der Zugang wird durch das Bohren des Felsenbeins bis zum Niveau der Lamina compacta des Sulcus sinus sigmoides und zum Lumen des Canalis semicircularis lateralis erreicht. Auf diese Weise ist es ohne Gefahr möglich, den ganzen Fasciculus acustico-facialis, die Gegend des Foramen jugulare, den N. IX, X, XI, V und VI sowie auch die laterale Fläche des Hirnstamms sichtbar zu machen. Unserer Meinung nach übertrifft der beschriebene Zugang den translabyrinthären Zugang durch den Mastoid.

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## FIRING MECHANISMS IN THE SINGLE VESTIBULAR NEURONS IN THE CAT

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**Abstract.** Spontaneous unitary discharges in nucleus vestibularis lateralis (NVL) neurons were studied in locally anesthetized cats. The mean  $\pm$  S.E. firing rate of spontaneous unitary discharges of NVL neurons was  $19.8 \pm 0.9$  Hz. About 12% of NVL neurons showed a random firing. The spontaneous unitary discharge rate of the gamma distribution at  $\lambda = 2$  was relatively low. However, the others were high. Patterns of peak interval time of the distribution had one peak and the interpeak interval time of those with two and three peaks were almost the same.

The physiological reaction of the vestibular nerve and its nuclei to caloric stimulation and sensory stimulation has been well documented (Adrian 1943; Fredrickson et al. 1966; Lowenstein & Sand 1940a, b; Matsuoka 1967, 1969, 1971; Shimazu & Precht 1965; Weber & Steiner 1965). However, there is still a paucity of data regarding the firing mechanisms of single neurons in the vestibular system. Most investigators who examined the evoked activity of single neurons have commented on the background firing rate, though usually briefly (Adrian 1943; Lowenstein & Sand 1940a, b; Fredrickson et al. 1966; Matsuoka 1967, 1969, 1971, 1972a, b, c; Matsuoka et al. 1971, 1972, 1973; Shimazu & Precht 1965; Weber & Steiner 1965).

Spontaneous activity is a striking feature of the sensory portion of the nervous system. Long after specific stimuli have been discontinued, neurons in various afferent areas continue to discharge. Appropriate terminology for this firing has not been formulated. The term "spontaneous" is widely used and the difficulties with its use are generally appreciated. Yet it appears preferable to other terms such as "background," "maintained" or "on going."

Lowenstein (1940) and Adrian (1943) reported that the frequency of spontaneous unitary discharges in the vestibular nerve and vestibular nuclei is very high even in barbiturate-anesthetized animals. However, Shimazu & Precht (1965) and Matsuoka (1967 and 1969) reported the frequency of spontaneous unitary discharges to be relatively low and some of those neurons are completely silent. In the present experiments, spontaneous unitary discharges were recorded in the nucleus vestibularis lateralis (NVL) of locally anesthetized and decamethonium-immobilized cats. Spontaneous unitary discharges were described in terms of their firing rate.

### METHODS

Twenty adult cats of both sexes, weighing 2 to 4 kg, were used. All surgery was performed

Gamma distribution at  $\lambda = 2$

The distribution pattern F is the theoretical gamma distribution pattern at  $\lambda = 2$  and formula d programmed in Discussion.

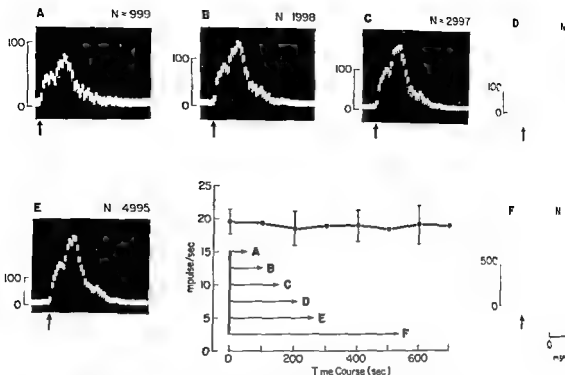


Fig. 1 Reproducibility of the time histogram of a lateral vestibular neuron. Total number of spikes in each distribution varied between 999 and 9990. Note that the mean firing

rate and the shape of the histogram from A to F are the same. Calibration as noted.

under diethylether-oxygen anesthesia. The trachea and the right femoral artery and vein were cannulated. The mucous membrane of the middle ear cavity of the right side was removed through an approach via the right bulla. A small concentric bipolar electrode was inserted into the lateral ampullar nerve with the aid of a microscope.

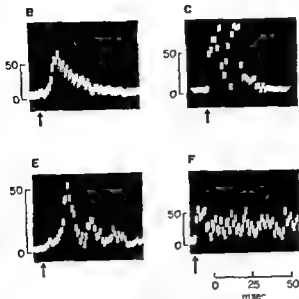
The recording microelectrode was gold-tipped stainless steel wire with an electrical resistance between 0.75 and 10 M $\Omega$ . The recording electrode was inserted into the right vestibular nuclei, primarily NVL, with the aid of a stereotaxic instrument, using the stereotaxic coordinates of a brain atlas (Snider & Niemer, 1961).

The recording microelectrode was connected through a cathode follower to a Grass P-5 amplifier and the potentials displayed on a Tektronix dual beam oscilloscope. An amplitude discriminator was used to convert amplitude and duration (TMC Model 605, 606). The output was fed into a CAT 400B computer

and date printer (TMC, Model 500A) which yielded an on-line interspike interval histogram of single unit activity. All data played on the oscilloscope were photographed on Kodak plus X film using a Grass camera. The data from the CAT 400B computer were also displayed on a dual beam oscilloscope and recorded on Polaroid film.

One sec or 625 msec were adopted as sweeping times of 400 data points on the interval time histogram. Each point contained 1 msec or 2.5 msec of interval time. After operative procedures were completed, 0.1% lidocaine was applied at all wounded edges after which the animal was immobilized with decamethonium (1.0 mg/kg/hr i.v.) and artificially ventilated. Body temperature was maintained at 36° to 38°C by means of an automatic heating pad (German Rupp Industries, Inc., Model K-13).

At the end of each experiment, 22.5 V d.c. was applied to the recording electrode so that the site of recording could be confirmed.



2 Types of interspike interval time distributions of ventral lateral vestibular neurons (total spikes in each distribution=999) A gamma distribution at  $\lambda=2$  B

monomodal distribution C bimodal distribution D exponential distribution E trimodal distribution and F rectangular distribution. Calibration as noted

ologically. A detailed description of the experimental procedures can be found in a previous paper (1973).

## RESULTS

Spontaneous unitary discharges from NVL of anesthetized animals were mainly biphasic but monophasic spikes were observed occasionally. The majority of the neurons had 0.2 to 1.5 mV in amplitude ranging from 10 to 70 Hz. The mean frequency  $\pm$  S.E. of neurons was  $19.8 \pm 0.9$  Hz. Silent neurons in the low frequency neurons below 0.1 Hz were not studied.

It is difficult to satisfy the mathematical requirements for a stationary process in view of the relatively limited size of sampling of spontaneous activity. It was necessary that there was a uniform development of the histogram over the period of analysis. The shape of the histogram remained constant. As shown in Fig. 1, the mean rate of the discharge in the period analysed was stable. The histogram of intervals accumulated uniformly,

although the total number of spikes in each distribution was different. As there was no difference in area size between 999 to 1990, the former was chosen for convenience. The interval time histogram analysed at channel widths of 1.56 msec or 2.50 msec was classified into the following six types, depending upon the shape: group A showing gamma distribution at  $\lambda=2$  and histogram of interspike interval of the NVL neuron fits the theoretical gamma distribution at  $\lambda=2$  (Fig. 2A), group B with a monomodal distribution has a peak in a histogram and the peak interval time of this histogram is 12.5 msec (Fig. 2B), group C with a bimodal distribution has two peaks and the peak interval times of this histogram were 10.0 msec and 22.5 msec (Fig. 2C), group D with an exponential distribution fits the theoretical exponential distribution (Fig. 2D), group E with a trimodal distribution has peaks and the peak interval times of this neuron were 15.0 msec, 32.5 msec and 45.0 msec (Fig. 2E), group F with a rectangular distribution has no peak, which means that the firing interval is not regular, but random (Fig. 2F).



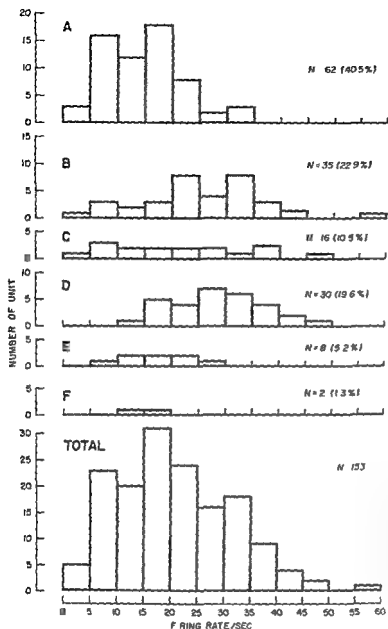


Fig 3 Relationship between the firing rate histogram and the distribution of spontaneous unitary discharges of larval neurons. Each histogram shows the frequency distribution of the mean discharges comprising families of spikes. The histogram for the entire discharges obtained by pooling the data is shown at the bottom.

About 40% of the neurons exhibited the pattern of *gamma* distribution at  $\lambda=2$ . Of 153 neurons recorded from the NVL, the number of neurons showing *gamma* distribution at  $\lambda=2$ , distribution with monomodal, bimodal, exponential distribution and distribution with trimodal, was 62 (40.5%), 35 (22.9%), 16 (10.5%), 30 (19.6%), and 8 (5.2%), respectively. The two remaining neurons (1.3%) showed a rectangular distribution pattern (Fig 3). Sixty-two out of 153 neurons showed a *gamma* distribution at  $\lambda=2$ . The mean firing rate of this

group was relatively low, ranging from 35.0 Hz and  $13.0 \pm 0.8$  Hz (S.E.). Thirty out of 153 neurons had a monomodal distribution. The mean firing rate  $\pm$  S.E. of this group was  $25.2 \pm 1.5$  Hz and ranged from 0.1 to 55.0 Hz. The peak interval time of this group was 10.0, 12.5, 15.0, 20.0 and 30.0 msec (Fig 4). Sixteen out of 153 neurons revealed a bimodal distribution. The mean firing rate of this group was  $21.3 \pm 2.7$  Hz. The peak interval time is shown in Fig 4B. The first peak of this group had a very short interval

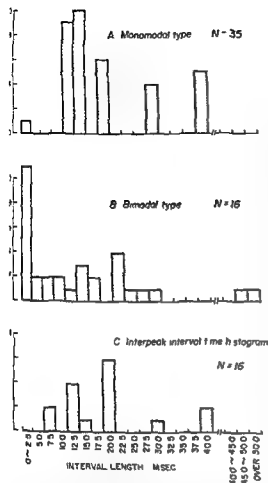


Fig. 4. Predominant interspike time histogram of various types of vestibular lateralis neurons. Note that the length of interpeak time interval with monomodal distribution is the same (A and C) as with bimodal distribution.

The interpeak interval time of each peak is shown in Fig. 4C. This pattern was almost the same as the peak interval time histogram observed in the monomodal (Fig. A). Thirty out of 153 neurons showed an exponential distribution. The mean firing rate  $\pm$  S.E. of this group was  $28.5 \pm 1.6$  Hz. Eight out of 153 neurons had a trimodal distribution. The mean firing rate  $\pm$  S.E. was  $17.5 \pm 1.6$  Hz. The peak and interpeak interval times are shown in Table 1. The pattern of this interpeak interval time was the same as observed in monomodal distribution. Only two out of 153 neurons showed

a rectangular distribution. The unitary discharge rate of this group was relatively low and the discharges were fired at random.

## DISCUSSION

The mean firing rate  $\pm$  S.E. of spontaneous unitary discharges in NVL of locally anesthetized cats was  $20.0 \pm 0.9$  Hz. This figure is almost the same as that described by Matsuoka (1967, 1969), who reported values of  $19.3 \pm 2.5$  Hz ( $N=62$ ) and  $21.4 \pm 0.6$  Hz ( $N=299$ ). A significant decrease in the frequency of the spontaneous unitary discharges of NVL neurons induced by administration of barbiturates has also been confirmed by Matsuoka (1967).

It is well known that neurons with an exponential distribution pattern fire at random and that the activity of neurons with a gamma distribution at  $\lambda=2$  can be explained by the feedback transmission process within a closed circuit. Hagiwara (1950), Naito (1966), Oomura et al. (1964 and 1967), Takaoni et al. (1968) and Sasa (1969). It is also well known that in the vestibular system the vestibular hair cells are excitatory to NVL neurons and the

Table 1. Peak and interpeak interval time in trimodal neurons ( $N=8$ )

Interval length (msec)	Peak interval time	Interpeak interval time
< 2.5	4	0
~ 5.0	0	0
~ 7.5	0	0
10.0	1	0
12.5	0	9
15.0	5	0
17.5	0	5
20.0	0	0
22.5	7	0
25.0	0	0
27.5	0	0
30.0	1	1
32.5	5	0
35.0	0	0
37.0	1	0
40.0	0	0
> 40.0	5	1
	24	16

NVL neurons are inhibitory (directly or indirectly to the vestibular hair cells). The recurrent collateral and cerebellum system to NVL neurons also has the same feedback system. In the present experiments, about 20% of the NVL neurons showed a pattern of exponential distribution which indicates that only 20% of the NVL neurons fired at random in the animal at rest, while the remaining neurons are associated with feedback mechanisms. About 40% of the neurons with the gamma distribution at  $\lambda=2$  were observed. The same distribution was observed in the nucleus tractus spinalis and nucleus sensorius superior (Takaori et al., 1968; Sasa, 1969). As shown in Fig. 3, the interval distribution of each of the input pulse trains is assumed to be a gamma distribution at  $\lambda=2$ .

$$P(T) = \frac{K a T^{a-1} e^{-KT}}{T(a)} \quad (0 \leq T < \infty)$$

where  $T$  = length of interval,  $p(T)$  = probability of interval length  $T$ , and  $K$ ,  $a$  are the adjustable constants. The important parameter determining the shape of the distribution is the constant  $a$ , the value  $a=2$  is chosen corresponding to the value obtained experimentally for peripheral cell discharges (Kuffler et al., 1957; Bishop et al., 1964). For simplicity,  $K$  is set equal to unity. This gamma distribution at  $\lambda=2$  is the shape of the assumed interval distribution for the impulse train of each excitatory input fiber (Bishop et al., 1964). In the present experiments, the proportion of these neurons was much greater than in the data from Fujita et al. (1968), who reported that the neurons in the NVL showed only a monomodal distribution. The peak interval times of the distribution were 8.4 msec, 24.6 msec, 26.3 msec, 41.9 msec, and 54.5 msec, respectively.

The peak interval time observed with the monomodal distribution was relatively longer than that in the data from Fujita et al. (1968). However, the pattern of this group was almost the same. The peak interval of this group had almost the same value of the interpeak interval time as was observed with the bimodal and

trimodal distribution. The excitatory feedback system in these neurons presumably accounts for these phenomena.

## ZUSAMMENFASSUNG

Die Spontanaktivität von Neuronen im Nucleus vestibularis Lateralis (NVL) von lokal narkotisierten Katzen wurde untersucht. Die durchschnittliche Entladungsfrequenz der Spontanaktivität von diesen Neuronen war 19.09 Hz. Die Spontanaktivitätsraten der Neuronen Gamma-Verteilung ( $\lambda=2$ ) zeigten waren relativ niedrig die der Neuronen aber hoch. Die Formen Spitzenintervallezeit (Kurven mit einer zwei und Spitzen) waren fast gleich.

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## EFFECTS OF CHOLINERGIC AGONISTS AND ANTAGONISTS ON NUCLEUS VESTIBULARIS LATERALIS UNIT DISCHARGE TO VESTIBULAR NERVE STIMULATION IN THE CAT

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**Abstract** Unit discharge in nucleus vestibularis lateralis (NVL) was studied in locally anesthetized cats. The mean firing rate  $\pm$  S.E. of spontaneous unitary discharges of NVL neurons was  $19.8 \pm 0.9$  Hz and that of spinal transected animals was  $18.0 \pm 1.6$  Hz. The mean firing rate of these neurons in animals with cerebellum ablation and the administration of scopolamine in a dose of 0.5 mg/kg i.v. was relatively low. After administration of physostigmine in a dose of 25  $\mu$ g/kg i.v. the firing rate was enhanced. The effects of physostigmine were antagonized by scopolamine. About 95% of NVL neurons activated by single electrical shocks to the ipsilateral vestibular nerve showed regular firing following administration of physostigmine. These neurons changed to a gamma distribution at  $\lambda=2$  following administration of scopolamine.

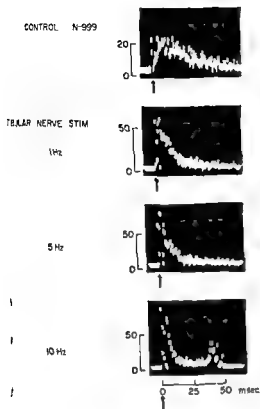
It has been demonstrated that neurons in the vestibular nuclei are excited by the local administration of acetylcholine and/or cholinesterase inhibitors such as physostigmine given parenterally (Weber & Steiner, 1965; Yamamoto 1967; Matsuoka et al., 1973). The histochemical localization of acetylcholinesterase (AChE) in the nucleus vestibularis appears complex. AChE levels are high in the lateral and superior vestibular nuclei, but low in the medial and inferior nuclei (Friede, 1966). In nucleus vestibularis lateralis (NVL) almost all of the AChE are contained in the cell bodies of Deiters' giant neurons. Shute & Lewis (1960) and Ross (1969a, b) have also reported that AChE is present in the vestibular

ganglion of Scarpa. Matsuoka et al. (1972, 1973) have also reported that the content of cholinesterase, AChE, choline acetyltransferase and total cholinesterase was higher in NVL than in the vestibular nerve including the vestibular ganglion of Scarpa. Steiner & Re (1965), Yamamoto (1967) and Matsuoka et al. (1973) have reported that NVL neurons are activated by vestibular nerve stimulation and are also stimulated by the administration of acetylcholine. The present report describes studies on the firing mechanisms of the NVL neurons following vestibular nerve stimulation and the intravenous (i.v.) administration of cholinergic agonists and antagonists.

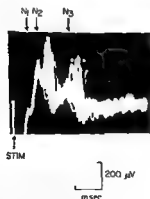
### METHODS

Twenty-five cats of both sexes weighing 2.4-4.0 kg were previously confirmed to be normal regarding voluntary movement. The operative procedures and recording techniques were similar to those described previously in lidocaine locally anesthetized and decathionium immobilized cats. For vestibular nerve stimulation a small concentric bipolar electrode was inserted into the lateral ampullary nerve with the aid of a microscope after which it was fixed with dental cement.

A



B



Changes in interspike interval distribution following vestibular stimulation. A total of 999 discharges were recorded from the same single neurons. A ipsilateral vestibular nerve was stimulated with single shocks 5 and 10 of 0.05 sec duration and 3.5 V. Evoked potential and evoked unitary discharges are illustrated. Twenty sweeps were superimposed. Note that the evoked unitary discharges are elicited on the monosynaptic potential ( $N_2$ ) and  $N_2$ - $N_3$  synaptic potential ( $N_3$ ). Time base and voltage calibration are as shown in the figure.

The administration of physostigmine in a dose of 25  $\mu$ g/kg and scopolamine in a dose of 0.5 mg/kg was given i.v. A square pulse of 0.05–0.1 msec duration at 1, 5, and 10 Hz was applied to the ipsilateral vestibular nerve. The statistical validity of the distribution pattern of the histogram was determined using a Chi square test. Changes in unit firing rate caused by electrical stimulation of the vestibular nerve were determined following the formula described below and were confirmed according to the Chi square test using a criterion of  $p < 0.05$ .

$$\text{If the firing rate is below ten, } \chi^2 = \frac{(F_0 - F_e)^2}{F_0 + F_e}$$

$$\text{If the firing rate is over ten, } \chi^2 = \frac{(F_e - F_0)^2}{F_0 + F_e}$$

$F_0$  = total number of the spontaneous unitary discharges for 5 sec before stimulation,  $F_e$  = total number of the unitary discharges for 5 sec during and after stimulation. In some animals, spinal section and ablation of the cerebellum were performed under diethyl ether-oxygen anesthesia.

## RESULTS

Spontaneous unitary discharges from NVL in locally anesthetized, decamethonium immobilized artificially ventilated animals were observed. These data have been reported previously (Matsuoka et al., 1973). One hundred and fifty-three neurons in NVL were classified according to the distribution pattern. The interval distribution of unitary discharges in NVL consisted of the following six groups: gamma distribution at  $\lambda=2$ , monomodal, bimodal, trimodal, exponential, and rectangular distributions. Thirty-eight of the neurons tested were responsive to the vestibular nerve stimulation with a frequency of 1, 5, and 10 Hz. The neurons responsive to vestibular nerve stimulation were classified into two responses: facilitatory and inhibitory types. The facilitatory types could be subdivided into three patterns: type I, in which the firing rate increased

Table IV Distribution pattern of spike intervals for the same neurons before and after administration of physostigmine (25  $\mu$ g/kg i.v.) and scopolamine (0.5 mg/kg i.v.)

Control	After admin of physostigmine	After admin of scopolamine	No of neurons
Gamma distribution	Distribution with monomodal	Gamma distribution	11
Distribution with monomodal	Distribution with monomodal	Gamma distribution	4
Distribution with bimodal	Distribution with monomodal	Gamma distribution	2
Exponential distribution	Distribution with monomodal	Gamma distribution	2
Total			19

same neurons before and after administration of physostigmine and scopolamine is summarized in Table IV. The gamma distribution at  $\lambda=2$  and the bimodal and exponential distribution changed to the monomodal distribution pattern after administration of physostigmine. These neurons also changed to the gamma distribution pattern at  $\lambda=2$  following scopolamine.

Spinal cord transect at the  $C_1$  to  $C_2$  level did not change the spike interval distribution. The mean firing rate  $\pm$  S.E. of this group was  $18.0 \pm 1.6$  Hz, which is almost the same as the control (Table V). The pattern of spike interval

after ablation of the cerebellum is summarized in Table VI. The percentage of neurons showing a gamma distribution at  $\lambda=2$  fell to 2.9% while neurons showing monomodal, bimodal, and exponential distributions increased 38.6%, 31.4% and 27.1%, respectively.

## DISCUSSION

All NVL neurons analysed were responsive electrical stimulation of the ipsilateral vestibular nerve at 1, 5, and 10 Hz. The facilitator type dominated. These results are in agreement with those described by Adrian (1943), Lowenstein & Sand (1940a, b), Shimazu, Precht (1965) and Matsuoka (1967 and 1968). The spontaneous unitary discharges of NV neurons showed a marked increase in the firing rate after i.v. administration of physostigmine. On the other hand, scopolamine reduced NV neuronal firing considerably. These data parallel the data of Weber & Steiner (1964), Yamamoto (1967) and Matsuoka et al (1971). The difference between physostigmine and scopolamine was confirmed by observing the time course of the responses of the same neurons by giving the drugs in sequence.

All 19 neurons classified into three facilitatory types change their firing into monomodal distribution after administration of physostigmine. They then changed into

Table V Distribution pattern of spike intervals after spinal section ( $C_1-C_2$ ) in the nucleus vestibularis lateralis

Discharge rate (Hz)	Gamma distribution	Distribution with		Exponential distribution	Number of neurons n (%)
		Monomodal	Bimodal		
0-5	4	0	0	0	4 (6.2)
5-10	7	0	2	0	9 (13.8)
10-15	9	1	1	1	12 (18.5)
15-20	8	2	2	1	11 (16.9)
20-25	2	5	4	3	14 (21.5)
25-30	5	2	1	2	10 (15.4)
30-35	1	0	0	1	2 (3.1)
35-40	0	0	0	0	0
40 <	1	2	0	0	3 (4.6)
Total	35 (33.8%)	12 (18.5%)	10 (15.4%)	8 (12.3%)	65

Mean firing rate  $\pm$  S.E. =  $18.0 \pm 1.6$  Hz ( $N=65$ )

Table VI Distribution of spike intervals after ablation of the cerebellum

charge	Gamma distribution	Distribution with		Exponential distribution	Number of neurons n (%)
		Monomodal	Bimodal		
1	0	5	0	8	13 (18.6)
10	2	13	15	9	39 (55.6)
15	0	5	7	2	14 (20.0)
20	0	2	0	0	2 (2.9)
25	0	2	0	0	2 (2.9)
30	0	0	0	0	0
35	0	0	0	0	0
total	2 (2.9%)	27 (38.6%)	22 (31.4%)	19 (27.1%)	70

Mean firing rate  $\pm$  S.E.  $8.1 \pm 1.9$  Hz ( $N=70$ )

gamma distribution at  $\lambda=2$  following scopolamine. The dominant distribution pattern of the spike interval after administration of physostigmine was the monomodal, while the distribution of spike interval after administration of scopolamine was the gamma distribution at  $\lambda=2$ . The distribution pattern of spike intervals after spinal section was almost the same as in intact animals. On the other hand, the dominant pattern after ablation of the cerebellum was of the exponential type. These results support the assumption that neurons in NVL behave similarly to those in which the vestibular nerve is stimulated following the administration of physostigmine.

It is well known that neurons with an exponential distribution pattern fire at random. The activity of neurons with a gamma distribution at  $\lambda=2$  is best explained by a feedback transmission process within a closed circuit (Iatsuka et al. 1975). Therefore 30% of the NVL neurons fired at random following ablation of the cerebellum. The relatively large number of neurons in the NVL appears to be associated with such feedback mechanisms both in the control and the spinal transected groups.

Apparently spontaneous unitary discharges recorded from NVL fire with some feedback system. In the present experiments, NVL neurons responded most effectively to electrical stimulation of the vestibular nerve at frequencies of 1.5 and 10 Hz.

The time interval histogram of spontaneous unitary discharges in about four-fifths of NVL neurons showed a gamma distribution at  $\lambda=2$  pattern. The monomodal, bimodal and trimodal distribution and the majority of the spike intervals in these types of neurons were in the order of 25 msec and 50 msec. The peak interval time of the monomodal distribution and the interpeak interval time of the bimodal and trimodal distribution was in the order of 2.5 msec, 10 msec, 20 msec, 30 msec, and 40 msec respectively. There are also several types of closed circuits in these feedback systems and a short interval less than 2.5 msec revealed a closed circuit in the same NVL. The role of the other reflex influences of physostigmine and scopolamine given parenterally needs to be studied further. Nevertheless, the present study indicates an important cholinergic mechanism in relation to their portion of the vestibular system.

## ZUSAMMENFASSUNG

Das Entladungsmuster von Neuronen im Nucleus Vestibularis lateralis (NVL) wurde an Katzen in Lokalanästhesie untersucht. Die mittlere Spontanaktivität dieser Neurone betrug  $19.8 \pm 0.9$  spikes/sec beim normalen Tier und  $18.0 \pm 1.1$  spikes/sec bei Tieren mit spinaler Durchtrennung. Die mittlere Entladungsrate dieser Neurone war relativ niedrig bei Tieren mit abgetragenen Cerebellum und auch nach Verabreichung von 0.5 mg/kg Scopolamin (i.v.). Nach Eingeben von 25 µg/kg Physostigmin (i.v.) fand eine Erhöhung der mittleren Entladungsrate statt. Scopolamin hob den Effekt von Physostigmin auf Ca. 95% der NVL.



Neurone die durch elektrische Einzelreize des ipsilateralen N. Vestibularis aktiviert wurden zeigten normale Entladungsraten nach Eingeben von Physostigmin. Diese Neurone wurden durch Scopolamin zu einer Gamma-Verteilung ( $\lambda=2$ ) verändert.

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## THE RELATIONSHIP BETWEEN THE NUCLEAR DNA CONTENT IN SMEARS OF ASPIRATES AND THE PROGNOSIS OF MUCOEPIDERMOID CARCINOMA

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**Purpose** Cytophotometric analysis of nuclear DNA content was performed in a series of mucoepidermoid carcinomas with an extreme difference in the clinical course which was observed during a follow-up period of 13 years. The prerequisite for such a study was the development of a method which made it possible to study nuclear DNA content in tumour cells obtained at the time of diagnosis, i.e. 6-13 years ago in the present material. A special cytochemical procedure with destaining of the original Giemsa stain, refixation and subsequent Feulgen staining of the smears of aspirates in the original cytological material was developed. The cytophotometric nuclear DNA analysis of tumour cells in smears of aspirate from mucoepidermoid carcinomas showed that higher DNA tumours (near triploid) had a worse prognosis when compared with near-diploid tumours. These data are supported by our previous findings that a shift from a near-diploid to a near triploid DNA content of the tumour cells was associated with the prognostic important property of invasive growth. Thus the cytophotometric nuclear DNA analysis of aspirated tumour cells seems to be a valuable information about the malignancy degree of an individual tumour, which is not possible to obtain only from the cytomorphological features.

It has been shown that invasive salivary gland tumours are characterized by a higher degree of abnormality with respect to the DNA content than the non-invasive tumours (Eneroth & Zetterberg, *In press*). Thus, the property of non-invasive growth was found to be associated with a diploid or near-diploid DNA content, whereas invasive growth was associated with a triploid or near-triploid DNA content. These findings suggest that the nuclear DNA content may be a relevant cell

criterion in the evaluation of the prognosis, as the invasiveness of a salivary gland tumour is supposed to be the most significant criterion in evaluating the clinical course (Blanck, 1974). The aim of the present investigation was to further study the idea about a suggested relationship between the nuclear DNA content and the prognosis of salivary gland tumours. The prerequisite for such a study was to find a method in determining the nuclear DNA content in cells of salivary gland tumours operated so long ago that the prognosis could be evaluated by a follow-up period long enough. The prerequisites for an analysis of the nuclear DNA content of tumours operated many years ago were present as the slides with the smears of aspirates from tumours were archived. In order to be able to use this cytological material for the quantitative cytophotometric DNA analysis a special cytochemical procedure was developed.

### MATERIALS AND METHODS

#### *Clinical material*

Five cases were selected from a group of patients with mucoepidermoid carcinoma treated at the Department of Otolaryngology during the period 1961-1968. Three of the patients (cases 1, 2 and 3) were alive and free of symp-

toms at the end of the follow-up period (1974) 9, 13 and 6 years, respectively, after the primary operation. The remaining two patients (cases 4 and 5) died with general metastases in the tumour disease 3 years and 3 months, respectively, after the primary treatment.

Cases 2 and 3 were histologically classified as highly differentiated non-invasive mucoepidermoid carcinomas, case 1 as a moderately differentiated carcinoma without evident signs of invasive growth, and cases 4 and 5 as poorly differentiated carcinomas with general invasive growth into surrounding tissues.

### Cytochemical procedures

The original air dried Giemsa stained cytological preparations, from which the primary diagnosis of the salivary gland tumours was based 5–13 years ago, were used for the cytophotometric DNA analysis. In order to be able to use this cytological material for the quantitative cytophotometric DNA analysis a special cytochemical procedure was developed, involving destaining of the original Giemsa stain, refixation and subsequent Feulgen staining. Over night treatment in absolute methanol efficiently removed most of the Giemsa stain. The small amount of Giemsa stain remaining after this procedure was completely removed during the acid Feulgen hydrolysis. After the methanol treatment the cells were refixed in 10% buffered neutral formalin for a period of 12–24 hours. This refixation proved to be important in stabilizing DNA during the acid Feulgen hydrolysis and minimize the intercellular variability in amount of Feulgen positive material. The cells were thereafter stained according to the Feulgen procedure involving acid hydrolysis at room temperature ( $+22^{\circ}\text{C}$ ) in 5 N HCL for the optimal time of 1 hour.

The cytophotometric measurements of stained cell nuclei were performed in a rapid scanning microspectrophotometer (Caspersson & Lomakka 1970; Lomakka 1965). Granulocytes found to be mixed with the

tumour cells in each of the analysed slides were used as control cells. The nuclei from the granulocytes contain DNA values corresponding to the normal diploid content (2.0 C) with variation in the majority of cells between 1.75 C and 2.25 C. Occasionally granulocytes with values up to 2.5 C were recorded. The value of 2.5 C was therefore used as an upper limit of the normal DNA content. The 2.5 C is indicated by the broken lines in the figure. All measured values were expressed in relation to their corresponding granulocyte controls and expressed in C units.

In order to test if any quantitative errors were introduced when small, strongly light absorbing objects were measured such as nuclei with a compact chromatin (e.g. granulocyte control cells), measurements were also performed off peak at 610 nm at which wavelength the extinction was only about 40% of that obtained at the absorption peak around 546 nm. Independent of cell type (granulocytes with a compact chromatin and tumour cells with a dispersed chromatin) the ratio between extinction values at 546 nm and extinction values at 610 nm was found to be constant. Therefore no errors of any quantitative significance were introduced when measuring small, strongly light absorbing cells with a compact chromatin. In addition extinction values at 546 nm above 0.5 were selected for the cells that had undergone the above described staining procedure. In representative parts of the Feulgen stained smears, i.e. the parts including the mucoepidermoid carcinoma cells, were selected for the cytophotometric analysis and marked on the slides.

### RESULTS

Five patients operated on for a parotid tumour histologically classified as mucoepidermoid carcinoma were followed up for a period 6–13 years. The five patients were selected according to the clinical malignancy of the tumours. In one group low grade malignancy

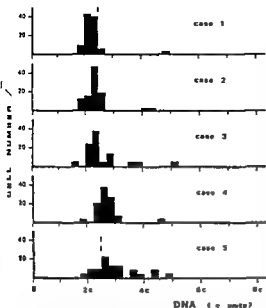


Fig. 1. Histograms of nuclear DNA content (Feulgen relative material) of individually analysed cell nuclei in the rapid scanning microspectrophotometer. The cell number in each histogram class is expressed in per cent of the total number of cells analysed. Between 50–100 tumour cell nuclei and between 10–30 granulocytes were analysed in each preparation. The measured values in this preparation were expressed in relative C-units. The 2C denotes the normal diploid DNA content determined as the mean granulocyte value. Mucoepidermoid carcinomas case 1, 2 and 3 were clinical low grade malignant tumours whereas case 4 and 5 were clinical high grade malignant tumours. The broken vertical line represents the upper extreme limit of the DNA value of granulocytes.

tumours were included (cases 1, 2 and 3). The patients in this group were all alive and free of symptoms at the end of the follow up period. The other group included two high grade malignant tumours (cases 4 and 5). These two patients had general metastases and died in the tumour disease 3 years and 3 months respectively after the primary treatment. Thus it could be studied whether a variability in the clinical malignancy was associated with any changes in the nuclear DNA content of the tumour cells.

From the histograms in the figure it is seen that most of the cell nuclei of the low grade malignant tumours (cases 1, 2 and 3) contained the diploid or near-diploid amount of DNA

(values around 2C). The fraction of tumour cells with abnormally increased DNA values ( $>2.5C$ ) was 9%, 25% and 34% respectively in case 1, 2 and 3. It is also evident from the histograms in the figure that the majority of cell nuclei of the high grade malignant tumours (cases 4 and 5) have DNA values corresponding to the hypo-triploid or triploid content (2.5C to 3C). The fraction of cells with an abnormally increased DNA value ( $>2.5C$ ) was 76% and 77% in these cases, i.e. considerably higher than in the low grade malignant tumours.

Thus an alteration of ploidy from a diploid or near-diploid DNA content in clinical low grade malignant tumours to a triploid or near triploid DNA content in clinical high grade malignant tumours was observed.

## DISCUSSION

Changes in nuclear DNA content of tumour cells have been found to be associated with the behaviour of the salivary gland tumour towards surrounding tissues (Eneroth & Zetterberg, *In press*). As this histological criterion (non invasive or invasive growth) has been found to be better than cellular polymorphism, mitotic rate, lymphoid stroma reaction etc. in predicting the prognosis in all types of salivary gland tumours (Blanc, 1974), it was suggested that the nuclear DNA content might be related to the prognosis of the tumour. This idea was further supported by the results of the present study, indicating a relationship between nuclear DNA content of the tumour cells and their malignancy, as judged from the clinical follow up study. The present study was based on an analysis of the nuclear DNA content of tumour cells from five mucoepidermoid carcinomas selected on the basis of an extreme difference in the clinical course. Three of the patients were alive and free of symptoms at the end of the follow up period and their tumours were therefore termed low grade malignant. Two patients died in the tumour disease within 3 years and the tumours of these patients were termed high-grade

toms at the end of the follow-up period (1974) 9, 13 and 6 years, respectively, after the primary operation. The remaining two patients (cases 4 and 5) died with general metastases in the tumour disease 3 years and 3 months, respectively, after the primary treatment.

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## GOBLET CELLS IN THE DEVELOPING HUMAN NOSE

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**Abstract** In a material of 56 foetuses and prematures in the 9th to the 30th week the nasal mucosa was removed *in toto* and stained with PAS-alcian blue PAS and the osmium whole mount methods. The development spread distribution and density of goblet cells were studied. Goblet cells start forming anteriorly in the nasal vestibule in the 13th week spreading backwards according to a given constant pattern in the course of the subsequent week. By the 30th week the goblet cells are present throughout the respiratory region but there are marked differences in density between the various areas. The highest density was found anteriorly and inferiorly on the septum and lateral wall and also in the inferior and middle meatuses. On the conchae the density is less at the junction to the olfactory region least. As a whole the density is still very low in foetuses and prematures and it must increase very considerably around the time of birth. The differences in density found in the present study are probably present also to some extent in children and adults. Formation of goblet cells is the final link in the process of differentiation into respiratory epithelium instituted 4 weeks earlier with the formation of ciliated cells.

It is well known that the epithelium of the respiratory region of the nose contains goblet cells but nothing definite is known about their distribution or density in the various parts of the nose. Together with the seromucous glands of the nose the goblet cells produce the nasal secretion which plays such an important role in the physiology and pathology of the nose. However any changes in goblet-cell density which may occur under pathological conditions—often described (Hajek, 1905, Oppikofer, 1907, Hansel, 1930, Eggston & Wolff, 1947, Messerklinger, 1958, Mygind et al., 1974) can be accurately assessed only

when their density in the normal mucosa is known.

The object of the present study was to ascertain the spread and distribution of cells during the development and growth of the nose. This ought to form a basis for studying the distribution in children and adults. We endeavoured to ascertain whether differences in density between the various regions were present already in foetuses and premature babies in order later to be able to interpret goblet-cell density in children and adults. There have been no special studies of the occurrence of goblet cells in foetal noses and no quantitative studies of their density in children and adults. According to Clara (1938) the differentiation from embryonic to pseudostratified ciliated epithelium has been completed by the 10th month. Bang (1964) finding goblet cells anteriorly in the nose in the 11th week believed that they appeared before the mucous glands. In the 17th week she found a high density of goblet cells anteriorly in the

### MATERIAL AND METHODS

The material comprises 56 foetuses and prematures in the age range 9th to 30th menarche week, assessed on the basis of crown-rump length (CR) and foot length according to Carter's tables (1921). After fixation and dissection from the outside, separating the nasal



Fig 1 Clustering of goblet cells. Several twin cells. Septum in 17th week. PAS-alcian blue whole mount,  $\times 350$ .

sa *in toto* from the surrounding tissue, one half of the nose was stained by a PAS-alcian blue whole mount method. The goblet cells stained a bright blue on a pale blue background. The other half of the nose was stained either by the PAS whole-mount method which stained the goblet cells red, or by the permanganate whole-mount method which does not stain the goblet cells, but the mucous glands. This latter method affords great help in

differentiating between groups of goblet cells and young gland primordia in the youngest fetuses. The advantages and disadvantages of these methods as well as the fine dissection at various ages have been described previously (Tos, 1966, 1970).

In each nasal half the spread of goblet cells was determined. The density was divided into three grades: High, moderate, and low, and the spread of the three density zones was



Fig 2 High density of goblet cells anteriorly on the septum in 16th week with marked PAS-alcian blue whole mount  $\times 60$ .



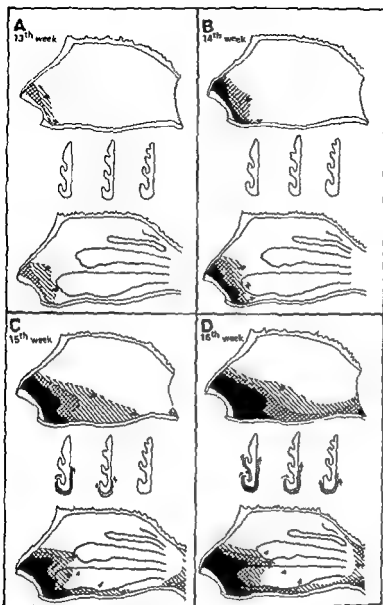


Fig 3 Spread of goblet cells at different ages. Top: Septum in the centre three vertical sections through the anterior pole, middle and posterior pole of the middle ear. Bottom: Lateral wall with the middle superior and supreme zones. Zones with high density black, with moderate density heavily shaded and with low density lightly shaded. Arrows indicate direction of spread, dotted arrows the direction beneath the conchae.

traced on a graph for each foetus. On six foetuses representing different age groups, the density was determined quantitatively, and the goblet cells were counted in Reichardt's projection microscope in a magnification of 500 $\times$ . According to foetal age and goblet cell spread, the counts were made in 100–230 fields measuring 0.01768 mm<sup>2</sup> equally distributed on the three density zones and the median density for each zone in the various age groups was calculated. Thereafter, parts of the whole mounts studied were cut into serial sections, stained with haematoxylin eosin and investigated.

## RESULTS

In the youngest foetuses, 9th and 10th week, which goblet cells had not yet formed, the epithelium was in the course of differentiation into pseudostratified ciliated. Anteriorly it was tall, pseudostratified, columnar with numerous ciliated cells, posteriorly shorter with fewer ciliated cells and hardly as mature as anteriorly. This indicates that ciliated cells differentiate before the goblet cells and that the process of epithelial differentiation starts anteriorly in the nose and proceeds in the anteroposterior direction.



Fig 4 Anterior half of nasal septum in 15th week. Clusters of goblet cells anteriorly in the vestibule. Mucous glands have spread farther back with the goblet cells. PAS-alc blue whole mount  $\times 30$ .

The appearance of goblet cells is the final link in the process of differentiation of the respiratory epithelium. In a few cells mucin granula form and gradually increase in quantity. In whole mounts this process is visible in the form of small faintly PAS alcian blue positive spots which gradually increase in size and staining intensity. The goblet cells are especially numerous in the younger foetuses as a rule in clusters of two (twin cells) four or more (Figs 1 and 2). This grouping may mean that mitotic division of the goblet cells can take place. The foetal goblet cells are large, distended with mucin.

#### Spread of goblet cells

Goblet cells start forming in the 13th menstrual week in a zone most anteriorly in the nasal cavity (Fig 3) and spread in the course of the 14th, 15th and 16th week backward especially along the nasal floor. Hence they spread upwards both on the septum (Fig 4) and beneath the inferior concha (Fig 5). From the rhinopharynx and the soft palate the goblet cells situated mainly in the mucosal sulci

force their way towards the nasal cavity, to join the anterior goblet-cell front on the nasal floor in the 16th week.

In the 17th week (Fig 5A) the goblet cell zone on the septum extends upwards (Fig 7) on the lateral wall up into the inferior meatus and backwards to the anterior third of the middle meatus and the medial surface of the inferior concha. By the 19th week (Fig 6B) the entire inferior meatus is lined with goblet cells. From the middle meatus they have spread up on the lateral surface of the middle concha and down over the anterior third of the medial surface of the inferior concha. From the rhinopharynx the goblet cells have spread forward in the posterior quarter of the middle meatus. Hence their spread continues down over the posterior pole of the inferior concha and up beneath the middle concha and further down on its lateral surface, lower edge and medial surface.

By the 23rd week (Fig 6C) the entire inferior concha and middle meatus have also become lined with goblet cells. From the entire extent of the middle meatus the goblet cells spread out on the lateral surface of the



Fig 5 Anterior half of the lateral wall in 15th week. The goblet cells reach to the anterior pole of the inferior concha; the glands farther back. PAS-alcian blue whole mount  $\times 30$ .

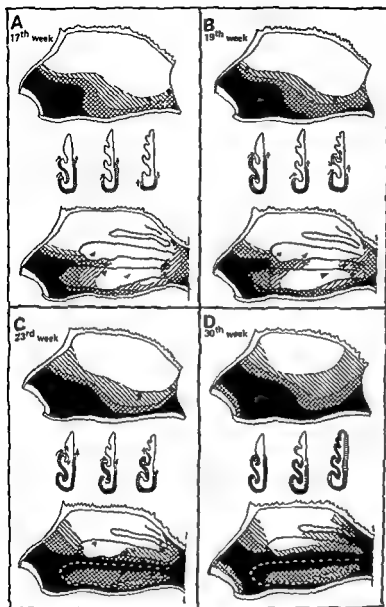


Fig 6 Spread of goblet cells at different ages. Explanation as in Fig 3

middle concha, down towards its lower edge, and up on the posterior half of its medial surface. Furthermore, an extension spreads from the rhinopharynx up towards the supreme concha, onwards in the superior meatus and hence up beneath the superior concha and down over the medial surface of the middle concha. Anteriorly the goblet cells spread over the anterior pole of the middle concha. On the septum the goblet-cell front reaches anteriorly almost to the nasal roof and posteriorly to the upper edge of the choanae.

By the 30th week there are goblet cells throughout the respiratory region, whose boundary runs on the middle concha in such a way that the entire posterior half and lower third of the medial surface, as well as the entire lateral surface, are lined with goblet cells. Moreover, the posterior two thirds of the superior meatus and posterior third of the superior concha as well as the entire supreme concha are lined with goblet cells.



7 Anterior half of septum in 17th week. High goblet density in the lower half, none in the upper. PAS-al blue whole mount  $\times 30$

### Density of Goblet Cells

Figs 3 and 6 show the distribution and spread of the three density zones in the course of development. Fig 8 shows the corresponding quantitative measurements for each zone and for each age group. Changes in density are best illustrated on the nasal septum. After the first goblet cells have formed anteriorly on the septum, the density in this area increases gradually and regularly during the subsequent 13 weeks (Fig 3), so that by the 16th week there is a fairly great density in the anterior end of the respiratory region (Figs 2, 3D and 8). After the goblet cells have spread during the subsequent weeks, to the posterior and anterior areas of the septum (Fig 6) the density in these sites also increases gradually, but not as markedly and not as rapidly as in the anterior parts. In the course of 7 weeks—from the 23rd (Fig 6C) to the 30th week (Fig 6D)—the border marking high density has not moved essentially upwards or backwards, and it is probable that at birth there is a considerable lower density in the upper part of the septum, especially at the limit to the olfactory region than in its lower part.

Similar appearances apply to the lateral wall, only far more complicated because of the conchae. In the anterior part the density increases, regularly and rapidly (Figs 3 and 8), and later it increases also in the inferior and middle meatuses, but not nearly as much as anteriorly, and especially not on the conchae, where it is still not particularly high in the 30th week (Fig 6D). It is low also at the junction to the olfactory region, and at birth it is probably lower here and on the conchae than in the meatuses.

The following factors influence the goblet cell density during the development of the nose: (1) The time of the first appearance of goblet cells, as the density is on the whole higher anteriorly, where goblet cells form first, than posteriorly. (2) Regional differences in density. Anteriorly on the inferior concha, superiorly in the nasal vestibule, and infero-posteriorly on the septum, where the first goblet cells appear as early as the 14th and 15th weeks (Figs 3B and C) density is still relatively low in the 30th weeks (Fig 6D). During this period, the density in these regions did not increase to the same extent as in other regions, and possibly it is also lower after birth in these regions. (3) Growth of the mucosa, whose area is constantly increasing. To keep pace with the growth of the area and maintain the attained density, new goblet cells have to form constantly. During the first weeks a rela-

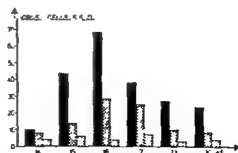


Fig 8 Median density of goblet cells (number/0.01768 mm² field) at different ages and different density zones: the extent of which is illustrated in Figs 3 and 6. Black, heavily shaded columns represent density zones showing high, moderate and low density respectively.

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## THE PATENCY OF THE MAXILLARY OSTIUM IN RELATION TO BODY POSTURE

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**Abstract** Eleven subjects were investigated concerning the effect of postural changes on the functional size of the maxillary ostium. The ostial size expressed as the percentage of the initial value decreased only slightly when the body position was altered from 90° (sitting) to 0° while the decrease between 30° and 0° (lying) was more pronounced. A cuff around the neck inflated to 25 mmHg gave a decrease corresponding to a value of 20° body position. Insufficient ventilation of the paranasal sinuses when lying down may result, especially in cases rarely having small ostia in the sitting position.

Patent ostia of the paranasal sinuses are essential for their normal functioning. Obstructions, for example, the maxillary ostium can be the first step in a process leading to an acute sinusitis. However, even when the ostium is patent the specific size is significant. When the ostium has a diameter less than 2.5 mm, indicating a relative ventilation insufficiency, the oxygen content of the maxillary sinus is lower than normal (Aust & Drettner, 1974). It is therefore of interest not only to evaluate the patency of the maxillary ostium, but also its functional size under various conditions.

One of the factors which may influence the functional size of the maxillary ostium is body posture. Winslow et al. (1934) found an increased swelling of the nasal mucosa in the recumbent position. Rundcrantz (1969) re-

ported that the nasal airway resistance increased in positions of 20° or less above the horizontal plane. This was found in healthy subjects as well as in patients with allergic and infectious rhinitis, although more accentuated in the latter patients. An increased airway resistance was also found during compression of the neck. Jonson & Rundcrantz (1969) showed that compression of the neck with 25 mmHg gave the same increase in pressure within the bulb of the internal jugular vein as the change in body position from sitting to recumbent. A change in the patency of the Eustachian tube according to posture was also found (Rundcrantz, 1969).

No studies of postural changes of patency of the maxillary ostium have been reported in the literature. Investigation must, in the first place, concern normal subjects, since about 50% of persons with allergic or infectious rhinitis already have obstructed ostia in the sitting or semi-recumbent position (Drettner & Lindholm, 1967).

### MATERIAL AND METHODS

Twelve subjects (9 men and 3 women) in the age range 20-32 were investigated. Eleven of them were healthy with no signs of allergy or infection and had normal rhinoscopy and radiographs of the paranasal sinuses. One sub-

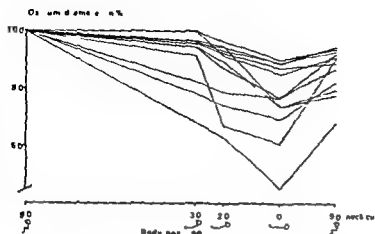


Fig. 1. The diameter of the maxillary ostium in different body positions, expressed as a percentage of functional size. The erect body position values are indicated in 11 subjects with the mean value illustrated by an interrupted line.

subject had a common cold with slight mucosal swelling of the maxillary sinus as revealed radiographically. One of the maxillary sinuses, usually the right, was punctured with a cannula of 2 mm diameter. The puncture was performed in the inferior meatus after local anaesthesia with a Xylocain® spray without adrenalin.

The patency of the maxillary ostium was checked by pressure recordings from the sinus during breathing, blowing and sniffing (Drettner 1965) when the patient was lying with the upper part of the body inclined 45° above the horizontal plane. When still lying in the same position another cannula was introduced in the same maxillary sinus close to the first and its patency was also checked in the same way as the first.

The functional size of the maxillary ostium was measured according to a principle published recently (Aust & Drettner 1974). One of the cannulas was used for intermittent insufflation of an airflow of 1, 2 or 4 l/min. The other cannula was used for recording the increase in pressure when the airstream entered via the sinus and left via the ostium. Calibration using the same cannula size was performed in a model in which the outlet representing the maxillary ostium was a tube with a length of 5 mm and varying diameters. The results were shown to be unrelated to the size of the maxillary sinus (Aust & Drettner 1974). The pressure increase obtained in the subject

was analysed using a nomogram from model experiments. In this way the functional size of the maxillary ostia in the subjects could be calculated.

The subjects were first investigated with the upper part of the body perpendicular to the horizontal plane. After a half minute in the same procedure was performed in positions 30° and 20° above the horizontal and then lying horizontally. Finally, in an erect sitting position the neck was compressed by an inflatable cuff. The pressure in the cuff was 25 mmHg (3.5 cmH<sub>2</sub>O) which was the same pressure used by Rundcrantz (1969).

## RESULTS

The subject with a common cold had an obstructed ostium during ordinary breathing and its size could therefore not be measured. In other subjects all had patent ostia. Their functional size decreased when the body position was changed from a sitting to a recumbent position. Fig. 1 shows the changes in the diameter of the functional size of the ostium expressed as percentages of the original diameter. The mean values were 95% at 30°, 87% at 20° and 77% at 0° (=recumbent position). The corresponding value at 90° (=erect position) was a cuff inflated to 25 mmHg around the neck was 87% of the original size, i.e. similar to the values measured at 20° body position.

The mean functional diameter of the ostium

Table I The functional size of the maxillary ostium—expressed as mm diameter and mm<sup>2</sup> cross section area—in 11 subjects investigated in different body positions and with a neck cuff inflated 25 mmHg. The volume of the maxillary sinus measured roentgenologically is also presented

Age	Maxillary sinus volume (ml)	Functional ostium diameter in mm					Ostium cross section area in mm <sup>2</sup>				
		90°	30°	20°	0°	90°+neck cuff	90°	30°	20°	0°	90°+neck cuff
27	15.5	11	08	07	05	08	10	04	04	02	05
31	10.1	11	11	11	10	11	10	10	09	08	09
29	7.8	18	—	17	16	17	25	—	23	20	23
27	27.3	19	18	17	14	15	28	25	23	15	17
23	20.2	23	21	21	20	20	40	36	34	30	31
27	24.9	23	18	17	16	19	42	25	23	20	28
20	13.8	24	22	16	14	22	43	36	19	16	36
21	27.5	26	22	21	20	24	53	36	33	31	45
27	24.9	28	27	26	25	26	62	57	53	49	53
25	26.2	34	34	31	29	31	92	92	76	66	76
32	23.5	56	54	53	44	48	246	229	221	152	181
25.8	20.2	25	24	22	19	22	48	43	36	29	37

in erect position was 2.5 mm (cross section 1.48 mm<sup>2</sup>) and the corresponding value in recumbent position was 1.9 mm (2.9 mm<sup>2</sup>), which was also the lowest mean value (Table I) measured in any position. The number of subjects who had an ostial diameter smaller than 2.5 mm was 7 in the erect position and 8 in the recumbent position.

Thus, only one subject who had an initial ostial diameter greater than 2.5 mm in the erect position had values below 2.5 mm in the recumbent position. However, most of the subjects had values smaller than 2.5 mm in the erect position.

## DISCUSSION

The injection of air into the paranasal sinuses is usually considered dangerous; it is worth stressing that the precautions against accidents were rigorous. After introduction of the cannula into the maxillary sinus, pressure recordings were performed to ensure that the cannula was not blocked and that the maxillary ostium was patent. The insufflation of air was performed for only a few seconds each time and the airflow was chosen to be as small as possible. No complications or discomfort were experienced during the air insufflation.

The mean size of the maxillary ostium in the present study (2.5 mm) was exactly the same as in a larger series (Aust & Drettner, 1974) and the series seems therefore to be representative for a normal population.

The present procedures for changes in body position and for neck compression were essentially the same as those used by Rundcrantz (1969). Thus, it was possible to compare the results concerning changes in the maxillary ostium with changes in the nasal patency and Eustachian tube function. It is also obvious that all such postural changes are caused by hydrostatic changes in the venous pressure. Patients with allergic or infectious rhinitis were excluded from the present study since they usually have obstructed ostia of the maxillary sinus. The changes in the size of the maxillary ostium in the present investigation were very similar to those reported by Rundcrantz (1969) concerning nasal patency. Thus, both the changes in nasal patency and in ostial size were less between 90° and 30° than between 30° and 0°. The compression of the veins of the neck with a cuff having a pressure of 25 mmHg resulted in a change in ostial size which corresponded to that occurring when the position was altered from sitting to the corresponding effect on nasal



compression was still closer to the horizontal position, according to Runderantz (1969)

When the measurements of the maxillary ostial size are compared with the investigations of the function of the Eustachian tube (Rundcrantz, 1969) it is obvious that the patency of the tube decreased considerably, sometimes to such an extent that the ventilatory capacity became insufficient when the patient changed from the erect to recumbent position. A similar tendency was found concerning the maxillary ostium, though remaining patent in all subjects who originally had patent ostia.

A functional ostial diameter of 2.5 mm is usually required for sustaining a normal oxygen level in the maxillary sinus. It is therefore of interest that only 4 of 11 subjects had such an ostial size in the erect position while in the recumbent position only 3 subjects maintained such a size. If these figures are representative of the whole population, it is obvious that insufficient ventilation of the sinus, with a tendency towards a decreased oxygen content is very common. The distribution of the diameters was large in the present series but it was similar to that in a recently published series comprising 37 cases (Aust & Drettner, 1974). Many apparently normal subjects thus have an ostial diameter which may be more or less insufficient for the maintenance of the apical oxygen content at a normal level. So far, no conclusive evidence can be presented for a direct correlation between low oxygen content and sinusitis. Still it is worth noting that an anatomical physiological disorder of the ostia of paranasal sinuses may be a predisposing factor for sinusitis.

The present patient population was primarily composed of normal subjects. It seems likely that the postural swelling of the mucosa in the maxillary ostium shown in normal subjects, may be at least as pronounced in patients with infectious or allergic rhinitis. Thus the frequent occurrence of obstructed ostia even in the erect position in these patients may be still greater in the recumbent position and

the ostial resistance may also be more pronounced among those who even in the erect position have obstructed ostia of the maxillary sinus or some other paranasal sinus.

Since the oxygen tension in the maxillary sinus is not only decreased when the ostium is obstructed but also influenced by the degree of obstruction (Aust & Drettnier, 1974) such an increase in the resistance of the obstruction during the ostium during bed rest may have a harmful effect, especially during an upper respiratory infection.

## ZUSAMMENFASSUNG

Elf Probanden wurden im Hinblick auf postoperative Grössenveränderungen der Maxillarest im unteren Kiefer untersucht. Der Ostiendurchmesser veränderte sich im Vergleich zum initialen Wert nur geringfügig bei Liegender und bei 30° (sitzend) zu 90° (stehend) dagegen fand sich eine deutliche Verengung im Bereich von 30° bis 90° (stehend). Beim aufrecht stehenden Probanden ergab sich am Hals angelegte mit 25 mmHg aufgeblasene Gummimanschette die gleiche Ostienverengung wie eine Lageränderung von 90° auf 20°.

Insuffiziente Ventilation der Nasennebenhöhlen bei liegenden Probanden kann auf Östern zurückzuführen sein die bereits in aufrechter Position einen geringen Durchmesser aufweisen

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## OLFACTORY NEUROBLASTOMA

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A case of olfactory neuroblastoma (type I ory neurocytoma) in a 44 year-old man is described. The tumour grew extensively in the right nasal with involvement of the maxillary sinus and ethmoid region but was radically removed at operation. Prognosis and treatment are discussed in the light of literature.

Olfactory neuroblastoma is an uncommon tumour originating in the neuroepithelium of the olfactory area. More than 100 cases have been reported. The tumour is malignant and tends to metastasize and is sometimes fatal.

### REPORT OF CASE

The patient is a 44 year old electrician. Some years previously he had had a minor traffic accident and roentgen examination at that time had revealed a density in the right maxillary sinus. The finding was interpreted as hemorrhage in the maxillary sinus following the accident. No fracture was demonstrable. Unfortunately none of the roentgenograms from that occasion are available. It is not likely that the density in the maxillary sinus was in reality due to the tumour to be described. Repeat roentgenography was not made to check the finding. The patient had otherwise always felt well apart from an occasional cold. During the last year he has been troubled more and more by colds and nasal discharge. In May 1972 he was referred to

examination because of nasal obstruction and symptoms resembling those of right sided otitis media. The roentgen diagnosis was right sided pansinusitis without fluid levels and the patient was treated with antibiotics and nasal drops. The patient refused irrigation of the maxillary sinus.

At follow up some weeks later the patient no longer had any acute symptoms but the roentgenogram was of the same appearance as before (Fig. 1). Rhinoscopy now revealed a small polyp like formation in the right side of the nose, a formation which had previously escaped detection because of the swelling of the nasal mucosa. At evulsion the tumour was felt firmer than expected from an ordinary nasal polyp. Histological examination of the specimen showed the picture of olfactory neuroblastoma.

The patient was therefore admitted for further examination. Skull X ray showed no intracranial changes and no osseous alterations suggesting involvement of the dura. Tomography of the nasal sinuses revealed destruction of the medial wall of the right maxillary sinus and persistence of the massive cloudiness of the sinuses on the right side. The sphenoidal sinus and the frontal sinus had a normal air-content. Right sided carotid angiography did not show any changes in the intracranial vessels. Ophthalmologic and neurologic examination revealed no



Fig 4 Detailed picture olfactory neuroblastoma (type I olfactory neurocytoma) with regular cells often arranged in rows H & E  $\times 184$

The most common clinical symptoms are unilateral nasal obstruction, epistaxis, bulging of the tumour (presenting mass), pain and headache and excessive lacrimation (Skolnik et al, 1966)

It must be differentiated from all other forms of tumour of the upper part of the nasal sinus and the ethmoidal region and from lympho-

sarcoma and small-celled undifferentiated carcinoma (Obert et al, 1960) as well as from the rare forms of tumour such as extracranial meningioma (Lindstrom & Lindstrom 1969)

The radiosensitivity of olfactory neuroblastoma has probably been over-estimated in the earlier literature. These tumours are in fact not very radiosensitive (Gerard Merchant

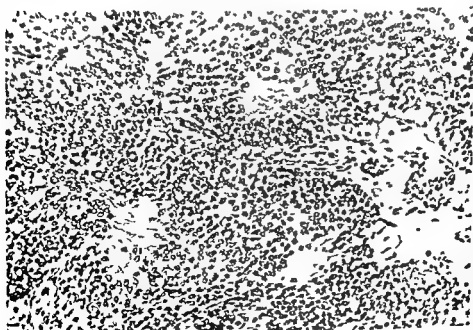


Fig 5 Part of olfactory neuroblastoma (type I olfactory neurocytoma) cells arranged in parallel converging rows and chromatin network in nuclei. Networks of fine fibrils between the cells H & E  $\times 178$

Chéau, 1965; Fitz-Hugh et al., 1965) and this is particularly to neurocytoma (Grahne,

Combined surgery and pre- or post-operative radiotherapy have been tried, but recurrences have nevertheless appeared in about half the cases. It is still too early to say anything about the value of cytostatic therapy. On basis of the 5-year-survival rate Skolnik (1966) recommend surgery of the primary lesion and reservation of radiotherapy limited with surgery for recurrences. In the present case treatment was planned according to principle.

## ZUSAMMENFASSUNG

Der Fall eines Olfactorus Neuroblastoms Typ I (neurocytoma) in der Nasenhöhle eines Mannes beschrieben. Weder klinisch noch histologisch konnte der Nachweis erbracht werden, dass der Tumor intrakraniell wirksam war. Der Tumor wurde durch Radikaloperation entfernt, es erfolgte in zweijähriger Observation kein Rezidiv. Die Prognose und die Therapie dieser seltenen Tumoren diskutiert.

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## MUCOSAL MALIGNANT MELANOMAS OF THE HEAD AND NECK

*With Special Reference to Cases having a Prolonged Clinical Course*

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**Abstract** A histological re-examination and re-classification of primary mucosal tumours of the head and neck region treated at Radiumhemmet and Karolinska sjukhuset during the period 1927-1970 revealed that 41 tumours were malignant melanomas. All these 41 tumours were located in the nasal cavity paranasal sinuses and oral cavity and not a single case of primary mucosal malignant melanoma was found in other locations of the head and neck region. In the present study the long term prognosis has been analysed. The follow up period was at least 5 years and ranged up to 48 years. It was found that mucosal malignant melanomas had a very poor prognosis with a five year survival rate of 17 per cent (7 of the total 41 cases) and a ten year survival rate of 7 per cent (3 of the total of 41 cases). The unpredictability of the clinical behaviour of this tumour type is elucidated by cases with a prolonged clinical course despite a primary relatively limited surgery, repeated local recurrences and regional lymph node metastases in an early stage of the disease. Thus there is always a never-ceasing risk of death in the tumour disease when once a malignant melanoma has occurred. For this reason a meticulous and long follow up of tumour patients is stressed and also the value of repeated surgery of local recurrences and regional lymph node metastases.

Although malignant melanoma is regarded as a highly malignant type of tumour, the unpredictability of its clinical behaviour has been pointed out. Thus cases of primary cutaneous malignant melanoma with a prolonged clinical course, despite regional lymph node metastases already at an early stage of the disease have been reported. However, mucosal malignant melanomas are found to carry a much poorer prognosis than cutaneous malignant melanomas (Allen & Spitz 1953, Moore & Martin 1955, Catlin 1967, Anneroth

et al., 1973). Thus the mean survival time shorter in mucosal than in cutaneous malignant melanomas, which can explain why reported cases of mucosal malignant melanomas with a prolonged clinical course (>10 years) cannot be traced in the literature with few exceptions (Catlin 1967, Freedman et al., 1973). In order to study the prognosis of mucosal malignant melanomas of the head and neck and to establish whether any of these tumours had a prolonged clinical course an extensive tumour material with a very long follow up period (5-48 years) was analysed.

### MATERIAL AND METHODS

The present study is based on 41 patients with primary mucosal malignant melanomas in the head and neck region treated at Radiumhemmet and the Department of Otolaryngology Karolinska sjukhuset during the period 1927-1970. In 24 of the 41 cases primary malignant melanoma was located in the nasal cavity and paranasal sinuses and the remaining 17 cases in the oral cavity. During the period 1927-1970 not a single case of primary malignant melanoma was found in mucosal locations of the head and neck region other than those mentioned above even though a few cases with metastatic deposits from primary malignant cutaneous melanomas were found in the mucous membranes of the

nx, larynx and trachea. Of the 24 cases the tumour involved the nasal cavity, 8 were involved both the nasal cavity and 5 of the paranasal sinuses, whereas 16 were limited to the nasal cavity without affecting the paranasal sinuses. In 8 of the 17 patients with malignant melanomas in the oral cavity, the tumour was located in the palate, in another 8 cases in the superior alveolus, and in one case the tumour was located in buccal mucosa.

The clinical follow-up study comprises all 41 patients operated upon during 1970 and continued until 31 January.

The postoperative follow-up examination of the patients was made at least once a year, both at Radiumhemmet and their local clinics, and the data on the clinical course were noted in the case records at Radiumhemmet. The observation period (4.18 years) was from the first histological verification of tumour.

The prerequisites for a histological re-classification exist, as the entire tumour material is held at the Institute of Tumour Pathology, Karolinska sjukhuset, partly as sectioned tumours and partly as paraffin-embedded material. The histological re-classification of material was made according to the criteria for malignant melanoma described by Eneroth and Berger (1973) and Anneroth et al. (1973). In all 41 cases, the histological re-examination shows definite malignant features with poorly differentiated cells, often with a great number of mitotic figures. In those cases where the tissues surrounding the tumour could be analysed, the tumour cells showed an invasive and diffuse infiltrative growth into the tissues, whereas the plasma-histamine reaction with single exceptions were markedly sparse. The histological diagnosis was based on the presence of melanin pigment and structure of the cells. Essentially three types of cell structure occurred. One was

composed of spindle, elongated, fusiform, usually richly pigmented cells of fibrosarcoma-like structures (19 cases), another of large, usually rounded polymorphous undifferentiated and poorly pigmented cells of carcinoma-like structures (14 cases), and the third a mixture of the fibrosarcoma-like and carcinoma-like types (8 cases).

## CLINICAL FINDINGS

Mucosal malignant melanoma of the head and neck region had no sex predilection (24 men and 17 women) in the series, and the age at the primary diagnosis of the tumour ranged from 34 to 80 years (mean 60 years). The symptoms varied with the site of the tumour and will not be discussed further. The duration of the symptoms before medical attention varied between one month and five years (mean 9 months). During the whole follow-up period local recurrences appeared in 21 of the 41 cases, and the first local recurrence occurred in all 21 cases within five years of onset.

### Metastases

Metastases appeared in 38 of 41 cases (93%) during the whole follow-up period. In 16 of the 41 cases metastases were demonstrable preoperatively or at the first treatment.

In all these 16 patients the metastases were located in the regional lymph nodes, i.e. there was no case of distant metastases diagnosed at the time of the first surgical intervention. Regional lymph node metastases appeared during the follow-up period in another 11 of the 41 cases, and all 11 cases within a period of 5 years from the first histological verification of the tumour (5 cases within 1 year, 4 cases between 1-3 years and 2 cases between 3-5 years).

Distant metastases appeared in 23 of the 41 patients after the primary treatment. The interval between the first operation of the tumour and the appearance of metastases is seen in Table 1. Further, this table shows that in 11 but 7 cases metastases appeared within 5 years of the first operation. In 4 cases

the histological re-classification was checked by G. Berger, Professor at the Institute of Tumour Pathology, Karolinska sjukhuset.

Table I *Primary mucosal malignant melanomas of the head and neck. Time interval between the first histological verification of the tumour and the appearance of distant metastases in 23 cases*

Time interval (years)	Number of patients
<1	10
1-3	6
3-5	3
>5	4
Total	23

distant metastases did not appear until after 5, 5½, 14 and 32 years, respectively. The two patients with the first appearance of distant metastases 14 and 32 years after the initial treatment of the tumour are unique, and the case history of these two patients will be given in detail (See case reports).

#### Survival

Thirty-nine of the 41 patients died during the follow-up period (Table II). It is evident from Table II that only 4 of the 41 patients did not die of the tumour disease during the follow-up period. One of the two patients registered as having died of intercurrent disease committed suicide seven months after the treatment of the primary tumour, but distant metastases had already developed. The other patient in this group died 4 years after the first surgical intervention from a cardiac infarction without any signs of the tumour disease. The two patients noted as living free of tumour disease were women and both were 34 years old at the initial treatment—one of them was living free of the tumour disease 16 years after radical operation of a malignant melanoma of the superior alveolar process, whereas the other patient was living free of the tumour disease 5 years after the operation of a mucosal malignant melanoma of the nasal cavity.

The survival time of the 37 patients who died in the tumour disease during the follow-up period is seen in Table III. It is obvious that the survival time after the initial treatment is

short. Thus, most of the patients had died within 3 years, and all but 5 patients within 5 years.

As the main purpose of the present study was to analyse cases of mucosal malignant melanomas with a prolonged clinical course, the two patients with a survival time of more than 10 years will be described in detail as case reports.

## CASE REPORTS

### Case 1

A 62-year-old woman was admitted to Karolinska sjukhuset in 1959, with the chief complaint of recurrent nosebleeds from the right nostril for two months. Examination revealed a soft hemangioma like tumour mass within the upper part of the right nasal cavity which appeared to have originated from the mucosa of the septum. A biopsy was made and it reported a malignant melanoma. Therefore a wide local cautery excision of the tumour was made. About one year after the primary excision regional lymph node metastasis appeared at the left side of the neck and therefore a left neck dissection was performed. The histological investigation established metastatic deposits of malignant melanoma in the lymph nodes. The patient did well until 1964 when a black coloured local recurrence was found and the patient underwent a Denker operation of the left maxillary sinus with trans-antral ethmoidectomy and spheno-tomy. It was found that the tumour had involved the bone so that the dura was laid bare. After this operation the patient was free from symptoms for three

Table II *A follow up study for 5-48 years of 41 patients with primary malignant mucosal melanomas of the head and neck*

Number of patients	Died of		Living	
	Tumour disease	Intercurrent disease	Free of tumour	4th tumour present
41	37	2	2	0

Table III Primary mucosal malignant melanomas of the head and neck. Survival time or first histological verification of the tumour in 37 patients who died of the tumour case

Survival time (years)	Number of patients
0-5	12
6-10	15
11-15	5
16-20	3
21-25	2
26-30	37

is but in 1967 a large greyish red mass of the nasal cavity and was found to be situated mainly on the lateral wall of the right nasal cavity. A Denker operation of the right maxillary sinus with transantral ethmoidectomy was performed. After this operation the patient had a very wide nasal cavity as the septum and the lateral walls of the nasal cavity were extirpated and the ethmoidal sinuses were evacuated bilaterally. After this operation there was no evidence of the tumour case until 1969 when a local recurrence was extirpated. During the period 1969-1973 cauterization and electrocautery of 11 local recurrences were performed. The local recurrences were small and of different colours such as red, brown or black and were almost always preceded by small bleedings from the nose. The patient did quite well during this period and the only trouble was crusts in the nasal cavity. During 1973 the local recurrences appeared more frequently than before and were also more extensive. In September 1973 the first manifestation of distant metastasis appeared in the form of a very big tumour in the small intestine which was resected.

In December 1973 a metastasis in the right ilium was extirpated and in February 1974 the patient was admitted to hospital with a heavy nosebleed. The whole nasal cavity was filled with a large grey blue bleeding tumour mass and on this occasion metastases in the mammary region were detected.

Thus after a 14 year period relatively tranquil tumour disease suddenly changed character and could not be controlled by further surgical activity. The patient died in April 1974 in the tumour disease—15 years after the first histological verification of the tumour.

### Case 2

A 37 year old man about three years before admission to his local hospital (1936) noticed a strip of brown discolouration of the upper alveolar mucosa. The brown patch had not bothered him so he ignored it for three years (until 1936) although it slowly enlarged. At this time he noticed a discolouration in the mucosa of the upper lip and the first examination of the patient revealed besides a non-elevated pigmented area (2×3 cm) in the upper alveolar mucosa and an indurated brown pigmented area (1×1 cm) in the mucosa of the upper lip to the right of the midline. No cervical lymph nodes were palpable. During the period 1936-1939 the pigmented areas were twice excised but unfortunately pathology reports are not available. Not until 1939 did a histological examination reveal that a pigmented tumour excised from the upper lip was a malignant melanoma. In 1940 the patient was admitted to Radiumhemmet and the physical examination revealed a blue discoloured scar in the mucosa of the upper lip and an irregular firm brown pigmented tumour on the upper alveolar mucosa. The tumour caused the patient no discomfort except for bleeding at the slightest provocation. A wide cautery excision of the tumour with the surrounding bone walls of the superior alveolar process and the pigmented area of the upper lip was performed. In 1941 neck dissection was performed on the right side and in 1942 on the left side. Metastatic deposits of malignant melanoma were found in regional lymph nodes in the specimens from both sides. After cautery excision and irradiation of a local recurrence in the upper lip in 1942 the patient was free of any further tumour disease until 1966 (24 years).



neck metastasis was extirpated on the right side, and the histological examination revealed malignant melanoma. Postoperatively the right area of the neck was irradiated with a total dose of 6000 r. Two years later (1968) cervical lymph nodes were palpable in the supra-clavicular region bilaterally and distant metastases appeared in the lungs. The patient died in August 1968 in his tumour disease 35 years after the first symptoms of the disease.

### TREATMENT

The 41 patients with mucosal malignant melanomas were treated with surgery, radiation therapy and chemotherapy in varying combinations. It is obvious of course, that the treatment could not have been uniform over such a long period as that during which the patients in the present series were treated (1927-1970). The most common practice has been surgery combined with irradiation. However, surgical procedures particularly in the early part of the period were often not sufficiently radical.

### DISCUSSION

The prognosis of mucosal malignant melanoma of the head and neck region is generally considered as very poor. Combining previously reported cases with those in their own study, Holdcraft & Gallagher (1969) found that the five year survival rate of malignant melanomas of the nasal and paranasal sinus mucosa was 11 per cent (24 cases of a total of 226 cases). Only one patient (0.5%) survived for ten years. In a series of 26 cases with malignant melanoma of the upper respiratory tract and oral cavity described by Moore & Martin (1955) only one patient survived for more than five years. From the review of the reports up to 1969 Holdcraft and Gallagher found that the five year survival rate varied from 6 to 17% in cases with mucosal malignant melanoma in the nasal cavity and

paranasal sinuses, which is the most common site for this tumour in the mucosal membrane of the head and neck.

The five year survival rate in the present series was 17% (7 of the 41 cases). Thus the five year survival rate of mucosal malignant melanomas of the head and neck is reported to be below 20%. Even in series described by Catlin (1967) and Fisman et al. (1973) a higher incidence of five year survival rate is found and a few cases with clinical course prolonged over more than years are reported, the long term prognosis of mucosal malignant tumours is extremely poor. Thus, the ten year survival rate was in the present series 7% (3 of 41 cases). One of three patients with a survival of over 10 years was alive and free of tumour symptoms at last observation 16 years after the first symptoms of the tumour, whereas the other two patients in this group died of the disease 15 and 35 years respectively after first symptoms of the tumour. The two mentioned cases of mucosal malignant melanomas are unique as regards their prolonged clinical course despite regional lymph node metastases in an early stage of the disease and the repeated local recurrence. Cutaneous malignant melanoma with this clinical course are, however, earlier in which can be explained by the fact that

there is a definite difference in the prognosis and therefore also in the survival time between patients with mucosal and cutaneous malignant melanoma (Allen & Spitz 1948; Moore & Martin, 1955; Catlin 1967; Eneroth & Moberger 1973; Eneroth, 1975a and b). The difference in prognosis between cutaneous and mucosal malignant melanoma is applicable to all skin and mucosal membranes (respiratory, alimentary and urogenital tracts). The reason for the prognostic difference between the mucosal and cutaneous malignant melanomas of the head and neck region discussed in the literature (Moore & Martin 1955; Mison & Friedmann 1955; Cron 1966) Delay in the treatment of the malignant

lanomas in the mucosa of the upper respiratory tract is regarded as the most prominent reason for the poor prognosis of these tumours. Whereas cutaneous malignant melanomas are usually clearly visible from an early stage, the mucosal malignant melanomas of head and neck region are frequently quiescent at the onset and produce insufficient symptoms to force the patient to seek medical advice in the early stages of the disease. Only when the condition at last is revealed, local surgical removal of the tumour is impossible. Furthermore, anatomical barriers oppose extreme radical surgery in lesions of upper respiratory tract compared to cutaneous lesions. Therefore, an increased knowledge of the initial symptoms of the mucosal malignant melanomas is the only means of making an early diagnosis possible, and essential for radical surgery of these tumours. Another reason for the difference in prognosis is between mucosal and cutaneous malignant melanoma can be a difference in the histological features. Thus as compared to cutaneous malignant melanomas of the head and neck (Eneroth & Moberger 1973) the malignant melanomas of the nasal cavity and paranasal sinuses (Eneroth, 1975a) and the maxillary cavity (Eneroth 1975b) seemed to be more highly malignant histologically with great cellular atypia, pleomorphism and higher incidence of mitosis.

In a comparison between primary cutaneous malignant melanoma and mucosal malignant melanoma of the head and neck region it could be shown that the mucosal lesions occurred in older age groups than did the cutaneous lesions. In the present study 80% of all patients were more than 50 years old at the first histological verification of the tumour. Comparing large series Allen & Spitz (1953) have shown that cutaneous malignant melanomas occurred only in 43% after the age of 50 years, whereas mucosal malignant melanomas of the nasal cavity and paranasal sinuses occurred in patients older than 50 years in 87% of the cases. In the literature only a few series of

years with mucosal malignant melanomas only occasionally are described.

Patients with mucosal malignant melanomas often have a very short survival time even though the primary lesion is small and the local surgery extensive. Local recurrences as well as metastases to regional lymph nodes, however, do not preclude the ultimate survival in malignant melanoma in contrast to most other forms of malignant tumours. This is elucidated by the two cases in the present study reported separately (case reports). The biologic behaviour of these two malignant melanomas is very curious. Despite relatively limited primary excisions of the tumours, repeated local recurrences and regional lymph node metastases in an early stage of the disease, these two patients survived for 15 and 35 years, respectively, before they died in the tumour disease. From these observations, the value of repeated surgical procedures of local recurrences and regional lymph node metastases is established. Furthermore, a careful follow up of these tumours is stressed by the fact that even after long intervals without symptoms (in one case 24 years) the tumour can recur. This indicates a never-ceasing death risk in the malignant melanoma disease no matter how long after treatment the patient lives. This probably represents quiescent disseminated disease held in check by a competent immunologic system (Freedmann et al., 1973).

## ZUSAMMENFASSUNG

Eine erneut aufgenommene histologische Untersuchung und Klassifikation von primären Schleimhauttumoren im Kopf- und Halsbereich, die in der Zeitspanne von 1927 bis 1970 im Radiumhemmet und Karolinska Sjukhuset in Stockholm behandelt worden waren, ergab, dass 41 Tumoren maligne Melanome waren. Alle diese 41 Tumoren waren auf die Nasenhöhle, die Nasennebenhöhlen und die Mundhöhle begrenzt. An anderen Stellen des Kopf- und Halsbereichs wurde kein einziger Fall von primären malignen Schleimhautmelanomen gefunden.

In der vorliegenden Arbeit ist die Langzeitprognose analysiert worden. Die Beobachtungsperiode war im

auf 48 Jahre. Das Ergebnis war, dass maligne Schleimhautmelanome eine sehr schlechte Prognose hatten, nämlich eine Überlebensrate von 17% für den Zeitraum von fünf Jahren (d. h. bei sieben von insgesamt 41 Fällen) und von 7% für den Zeitraum von zehn Jahren (bei drei von insgesamt 41 Fällen). Die Unmöglichkeit einer Voraussage des klinischen Verhaltens dieses Tumortyps veranschaulichen die Fälle mit verlängertem klinischen Verlauf trotz einer primär relativ begrenzten operativen Behandlung mit wiederholten Lokalrezidiven und regionalen Lymphknotenmetastasen in einem frühen Stadium der Erkrankung.

Es besteht daher bei diesen Tumoren immer das ständige Risiko eines tödlichen Ausgangs, wenn einmal ein malignes Melanom aufgetreten ist. Aus diesem Grunde ist eine äußerst sorgfältige und lebenslange Beobachtung von Tumorpatienten und der Wert wiederholter operativer Behandlung bei Lokalrezidiven und regionalen Lymphknotenmetastasen besonders hervorzuheben.

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## INCREASED LYMPHOCYTE ATP-ASE ACTIVITY IN PATIENTS WITH CARCINOMAS OF THE ORAL CAVITY AND LARYNX

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**Abstract** Increased mitochondrial ATP-ase activity was found in circulating lymphocytes from 5 out of 8 patients with carcinoma of the oral cavity or oropharynx and from 1 of 10 patients with carcinoma of the larynx. In a majority of these cases changes in the ATP-ase activity paralleled the clinical result of treatment. It is suggested that determination of ATP-ase activity of circulating lymphocytes is of diagnostic value in patients with oral, oropharyngeal and laryngeal carcinomas as well as of prognostic value after treatment of patients.

Evidence has accumulated indicating that the small thymus dependent lymphocytes (the T-cells) are the main effector cells in the host's defence against growth of neoplastic cells (Humble et al., 1967, Bubenik et al., 1970, DiSario et al., 1971, Richters et al., 1971, Hellström et al., 1971a, Perlmann et al., 1972), although the T-cell non-dependent lymphocytes (the B-cells) also can participate in the target cell destruction (Henney et al., 1972, Perlmann et al., 1972). The killing takes place when the lymphocytes, presumably sensitized by circulating tumour-associated antigens, obtain close contact with the malignant cells (Humble et al., 1956, Wilson, 1963, Ax et al., 1968, Richters & Sherwin, 1974). It is not yet fully understood how the lymphocytes destroy the

cancer cells, but the process is thought to be initiated by activation of a membrane-bound ATP-ase followed by a series of intracellular biochemical reactions ending with a nuclear activation and transformation of the lymphocytes. The energy required for these reactions may be provided by hydrolysis of ATP and in previous experiments we have demonstrated the presence of an increased ATP-ase activity in circulating small lymphocytes isolated from patients with malignant tumours (Ellegaard & Dimitrov, 1972a).

The ATP-ase activity present in the supernatant of homogenized lymphocytes was found to be stimulated by  $Mg^{2+}$  and dinitrophenol and inhibited by oligomycin, but relatively independent of  $Na^+$  and  $K^+$  and insensitive to ouabain. This serves to characterize the enzyme as a mitochondrial ATP-ase and in a few experiments it was demonstrated that this enzyme activity is actually confined to the mitochondrial fraction of the cytoplasm and constitute a greater part of the ATP hydrolyzing capacity of the supernatant of a crude lymphocyte homogenate (Ellegaard & Dimitrov, 1972a).

The present report demonstrates that the ATP-ase activity of lymphocytes is increased in patients with carcinomas of the oral cavity and larynx and that this activity decreases upon successful treatment.

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Table I. Clinical data and lymphocyte ATP-ase activity before and 6-12 weeks after treatment in 8 patients with oral and oropharyngeal carcinomas

Initials	Age	Sex	Localisation of tumour	Tumour stage	Effect of treatment	ATP ase	
						before	after
T N	69	M	Oropharyngeal	$T_1N_0M_0$	Complete	222	104
J A P	74	M	Oral bottom	$T_2N_0M_0$	Complete	73	99
P L	70	F	Gingival	$T_2N_1M_0$	Incomplete	134	128
L O P	70	M	Tongue	$T_2N_0M_0$	Complete	543	174
O P O	85	M	Buccal	$T_2N_1M_0$	Incomplete	90	163
L T	62	M	Buccal	$T_2N_3M_0$	Incomplete	152	165
E M L	57	M	Oropharyngeal	$T_2N_3M_0$	Incomplete	119	300
K J	50	M	Oral bottom	$T_2N_2M_0$	Complete	389	66

## MATERIAL AND METHODS

The study included a control group of 50 normal healthy subjects of both sexes (age 22-88 years), a group of 10 patients (age 47-83 years) with larynx carcinomas, and a group of 8 patients (age 50-85 years) with oral or oropharyngeal carcinomas. All the cancer patients had proven histological diagnoses and were staged according to the UICC classification (Tables I and II). Before therapy none of the carcinomas showed distant metastases and were thus all staged  $M_0$ . One of the larynx carcinomas ( $T_3$ ) had spread to the regional lymph nodes ( $N_1$ ), while the remaining were small localised tumours staged  $T_1$  in 5 cases

$T_2$  in 4 cases. Of the oral and oropharyngeal tumours 3 were localised while 5 showed regional lymph node metastases staged  $N_1$  in 2 cases and  $N_2$  in 3 cases. The tumours were staged  $T_1$  in one case,  $T_2$  in 2 cases, and  $T_3$ - $T_4$  in the remaining cases. The lymphocyte ATP-ase activity was determined shortly before and again 6-12 weeks after treatment, in one case however, 30 weeks after treatment. In all cases therapy was given as high-voltage irradiation (6000 rad). In one case (K J), a hemimandibulectomy with resection of the regional lymph nodes was also performed. The result of irradiation therapy is given as complete when the primary tumour no longer was visible or palpable and no signs of metastases had developed upon follow-up 6-12 weeks later.

The lymphocytes were isolated as previously described (Ellegaard & Dimitrova 1972a) from 50 ml heparinized blood. In order to remove the most sticky population platelets, the blood was passed slowly through a 5 cm column of washed 0.1 mm glass beads in a 10 ml plastic syringe. This prevents disturbing platelet aggregations during the subsequent isolation of the lymphocytes with the Ficoll-technique of Boyum (1968). The harvested lymphocytes were washed in saline and residual platelets and red cells removed by suspending the cells in 5 ml 10% sucrose and centrifuging for 15 min at 150 g on a discontinuous sucrose gradient made up of 25% 16% sucrose and 4 ml 20% sucrose. In rare cases a final 5 min exposure to a 1% ammonium oxalate solution was necessary to lyse a few remaining red cells.

The intact lymphocytes were now counted suspended in cold 0.25 M sucrose (10<sup>6</sup> cells per ml) and sonicated for 2 x 15 sec under cooling. The resulting homogenate was spun at 1000 g for 10 min in a refrigerated centrifuge and the supernatant used for the ATP ase assay (Ellegaard & Dimitrova, 1972a). In this the liberation of inorganic phosphate ( $P_i$ ) from the substrate, Tris-ATP, during a one hour incubation at 37°C is determined. The results were corrected for endogenous  $P_i$  and non-enzymatic  $P_i$ -release during the incubation. Double incubations and double determinations on each incubation were used throughout. The final re-

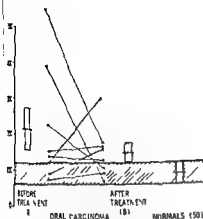


Fig. 1. Lymphocyte ATPase activity in 8 patients with oral and oropharyngeal carcinomas before and 6-12 weeks after treatment compared to the ATPase activity in 50 healthy controls. The columns express the group mean  $\pm$  1 SD and the shaded area the normal mean  $\pm$  1 SD.

are expressed as nmol  $P_i$  liberated per hour per mg protein of supernatant, the protein concentration being determined according to Lowry et al. (1951).

All reagents were of analytical grade. Ficoll was purchased from Pharmacia, Sweden and ATP from Sigma, USA.

## RESULTS

### Oral and oropharyngeal carcinomas

The staging of the tumours and clinical response to treatment of 8 patients with oral and oropharyngeal carcinomas are presented in Table I. Also shown are the lymphocyte

ATPase activities before and after treatment. The histological diagnoses were adenocarcinoma in two cases and squamous cell carcinoma in 6 cases. The ATPase activity before treatment averaged  $215 \pm 166$  (S.D.) nmol  $P_i$ /mg protein/hour, which is significantly higher ( $p < 0.02$ ) than the activity in lymphocytes from 50 normal controls ( $91 \pm 33$ ) (cf. Fig. 1). After radiation therapy the ATPase activity decreased to an average of  $149 \pm 72$  (S.D.).

Table I shows that in the four patients where the effect of treatment was deemed complete, the ATPase activity decreased dramatically in three, while the activity in the fourth patient remained unchanged within the indicated range. Among the four patients with an incomplete effect of treatment, the ATPase activity increased greatly in two cases and slightly in one case. This paralleled to a deterioration of the clinical condition and all these patients died within a year. The ATPase activity of the fourth of these patients remained unchanged at follow-up (134 vs. 128) and it is known that this patient was still alive one year after the investigation was completed.

### Laryngeal carcinomas

Table II shows the staging of the tumours and the clinical response to treatment in 10 patients with squamous cell carcinomas of the larynx as well as the ATPase activities before

Table II. Clinical data and lymphocyte ATPase activity before and 6-12 weeks after treatment in 10 patients with laryngeal carcinomas.

Patients	Age	Sex	Localisation of tumour	Tumour stage	Effect of treatment	ATPase	
						before	after
B. K.	50	M	Supraglottal	T <sub>2</sub> N <sub>0</sub> M <sub>0</sub>	Complete	167	123
M. K.	68	M	Supraglottal	T <sub>2</sub> N <sub>0</sub> M <sub>0</sub>	Incomplete	158	84
D.	58	M	Vocal cord	T <sub>2</sub> N <sub>0</sub> M <sub>0</sub>	Complete	79	177
K. N.	83	M	Vocal cord	T <sub>2</sub> N <sub>0</sub> M <sub>0</sub>	Complete	66	115
C.	65	M	Vocal cord	T <sub>2</sub> N <sub>0</sub> M <sub>0</sub>	Complete	233	97
M. N.	47	M	Vocal cord	T <sub>2</sub> N <sub>0</sub> M <sub>0</sub>	Complete	224	104
P.	69	M	Infraglottal	T <sub>2</sub> N <sub>0</sub> M <sub>0</sub>	Complete	68	103
R.	72	M	Infraglottal	T <sub>2</sub> N <sub>0</sub> M <sub>0</sub>	Complete	103	83
L. V. K.	80	M	Infraglottal	T <sub>2</sub> N <sub>0</sub> M <sub>0</sub>	Incomplete	117	200
A. M.	65	M	Infraglottal	T <sub>2</sub> N <sub>0</sub> M <sub>0</sub>	Complete	343	110

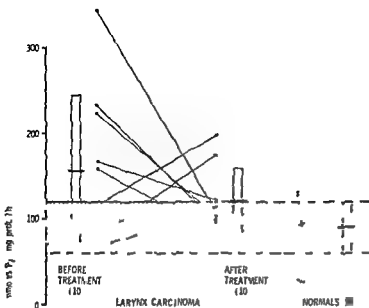


Fig. 2. Lymphocyte ATPase activity in patients with laryngeal carcinomas before and 6-17 weeks after treatment compared to the ATPase activity in 10 healthy controls. The columns express the group mean  $\pm$  S.E.M. and the shaded area the normal mean  $\pm$  1 S.D.

and after treatment. The average ATPase activity before irradiation was  $156 \pm 89$  (S.D.) which is significantly higher ( $p < 0.02$ ) than that of the controls.

In 8 patients the effect of treatment was evaluated as complete upon the immediate follow-up. In four of these cases (GBK, AC, TMN & CAM) the ATPase activity fell from high values to within the indicated range and in one case (AR) the activity fell slightly within the indicated range (Fig. 2). In the remaining three patients (VD, KKN & VP) the ATPase activity was definitely increased at follow-up. In one of these three cases (VP) a clinical relapse was later established while in the other two (KKN & VD) no sign of relapse has been noticed so far.

In 2 patients the effect of the treatment was evaluated as clinically incomplete. In one case the ATPase activity increased and in the other case the activity had decreased upon follow-up 12 weeks later.

### DISCUSSION

The reason for the elevated activity of lymphocyte mitochondrial ATPase in patients with carcinomas is not known. Stimulation of

the lymphocyte plasmamembrane by tumour antigens (Jehn et al. 1970) and phytohaemagglutinin A (PHA) (Craddock et al. 1971) results in increased protein, RNA and DNA synthesis and in blastic transformation of the cells. Stimulation by PHA leads to increased synthesis of phosphoproteins and to decreased intracellular concentration of ATP (Kleinsmith et al. 1966) as well as an increased activity of the mitochondrial ATPase (Ellegaard & Dimitrov 1972a). We suggest that these reactions might take place *in vivo* upon stimulation of the lymphocytes by circulating tumour associated antigens.

Measuring the lymphocyte ATPase activity might be of value as a diagnostic and in cancer. Previously only a few other biochemical tests for cancer have been developed and the most widely used of these has been the demonstration of increased serum concentrations of the carcinoembryonic antigen (Dykes & King 1972). This test is however almost invalidated by a high rate of falsely positive results (Martin et al. 1972). Aside from neoplastic diseases the lymphocyte ATPase activity has only been found significantly elevated in patients with ulcerative colitis and hepatic amoebiasis.

in the present series of patients with oral, pharyngeal or laryngeal carcinomas, the measured ATP-ase activity was significantly elevated compared to normal. From inspection of Figs 1 and 2, it is, however, evident that a normal pretreatment value of ATP-ase does not exclude the presence of these malignancies. For example, in one patient with oral carcinoma and lymph node involvement the ATP-ase activity was low before treatment and did not increase after therapy. The result of treatment was recorded as partially incomplete. A possible explanation for such a result might be sought in the fact that lymphocytes isolated from a malignant tumour or its regional lymph nodes in many cases show anergy against the tumour cells, probably due to immunological paralysis induced by blocking antibodies (Hellstrom et al 1971b, Nind et al, 1973).

Figs 1 and 2 also show that values above the indicated range (mean  $\pm$  S.D.) naturally are found in normal subjects. However, ATP-ase activities of more than  $2 \times$  S.D. above the normal mean value would be highly suggestive of the presence of malignancies and such values were found in one third of the patients with oral oropharyngeal carcinomas and in more than one half of the patients with laryngeal carcinomas, which under the circumstances makes the ATP-ase test a reasonable sensitive criterion.

At follow up 6-12 weeks after treatment, changes in ATP-ase activity in lymphocytes from patients with oral or oropharyngeal carcinomas corresponded to improvement or deterioration of the clinical condition and a pronounced rise in ATP-ase activity bore a grave prognostic sign. In case of the laryngeal carcinomas, the results at follow up were not unambiguous. In three of eight cases, where clinical evaluation showed that the treatment had been effective, the ATP-ase activity nevertheless increased significantly. In one of these cases, however, the clinical evaluation was regarded as a relapse was found on a later follow up. Whether the same result will be-

come evident for the two remaining patients, and the biochemical changes thus shown to precede the clinical evaluation of relapse of these low grade tumours, remains to be seen.

The ATP-ase activity of circulating lymphocytes has previously been found elevated in patients with carcinoma of the lung (Ellegaard & Dimitrov, 1972b), gastrointestinal tract (Dimitrov & Ellegaard, 1972b), uterine cervix (Ellegaard et al, 1974b) and the urinary bladder (Ellegaard et al, 1974a). The test also seems to be of diagnostic value in cases of carcinomas of the oral cavity and larynx and of prognostic value after treatment.

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### ZUSAMMENFASSUNG

Erhöhte mitochondriale ATP-ase Aktivität wurde in den Lymphozyten im Kreislauf bei fünf von acht Patienten mit Karzinom der Mundhöhle oder Oropharynx und bei fünf von zehn Patienten mit Carcinoma laryngis gefunden. Bei den meisten Fällen wurden Veränderungen in der ATP-ase Aktivität nach der Behandlung parallel zum klinischen Ergebnis der Behandlung gefunden. Es wird angenommen, dass die Bewertung der ATP-ase Aktivität der zirkulierenden Lymphozyten sowohl von diagnostischem Wert bei Patienten mit oralen oropharyngealen und laryngealen Karzinomen als auch von prognostischem Wert nach der Behandlung ist.

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## THYROPLASTY TYPE I (LATERAL COMPRESSION) FOR DYSPHONIA DUE TO VOCAL CORD PARALYSIS OR ATROPHY

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Based on the experimental results of thyroplasty type I which aims at medial shifting the vocal cord was performed on 8 patients with dysphonia: 6 with vocal cord paralysis and 2 with vocal cord atrophy. Surgery was conducted on either in- or out-patient and local anesthesia was used. Usually a rectangular incision was made on the thyroid cartilage at the level of the vocal cord and the fragment was depressed inward. A piece taken from the opposite side was used as a plug if necessary to enhance the effect of lateral resection of the vocal cord. The voice after surgery was generally satisfactory except in one case of traumatic vocal cord paralysis. Complications such as stridor or hemorrhage were nil. As surgical intervention inside the thyroid cartilage is minimal, fine and reliable adjustment of the vocal cord is possible during the surgery.

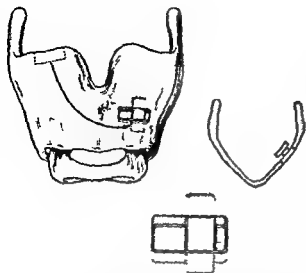
In our previous paper (Isshiki et al 1974) we reported experimental results of thyroplasties which aim at modifying the position and tension of the vocal cords. The experimental results on 14 dogs indicated the usefulness of various types of reformation of the thyroid cartilage for dysphonias artificially produced by the procedures such as sectioning the recurrent laryngeal nerve. From the functional viewpoints 4 types of thyroplasty were proposed: 1 lateral compression, 2 lateral excision, 3 relaxation (shortening) and 4 stretching (lengthening) of the vocal cord. The present paper describes the surgical technique and the results of thyroplasty type I (lateral compression). This surgery aims at shifting the vocal cord medially through deformation of

the thyroid cartilage and was performed on 8 patients with dysphonia: 6 with unilateral vocal cord paralysis and 2 with vocal cord atrophy.

### INDICATIONS

Any organic laryngeal disease which caused imperfect closure of the glottis during phonation without swelling or tumour of the vocal cord is an indication for thyroplasty type I. Most frequently it is indicated for dysphonia due to unilateral vocal cord paralysis. Thorough physical examination for detecting possible underlying disease is a prerequisite to any treatment of vocal cord paralysis. Furthermore, the patient should be followed up either on voice training and/or medication for at least 6 months after the onset of the disease in order to determine the underlying disease as well as the position and immobility of the involved cord. Oval glottal chink on phonation due to vocal cord atrophy or other diseases may be an indication for thyroplasty especially when the chink is large. Functional aspects or daily fluctuation of laryngeal findings and the degree of hoarseness should be taken into account prior to the decision of performing thyroplasty.

Manual test of the larynx is most useful for predicting the outcome. Manual compression



**Fig 1** The most typical thyroplasty type I. The rectangular incision on 10 mm×3 mm the upper margin of which is at the level of the vocal cord. A wedge may be inserted between the rectangular fragment and the remaining frame of the thyroid cartilage to enhance the compression effect when necessary.

of the bilateral thyroid alae at various points and/or bimanual approximation of the cricothyroid distance gives us a fairly good indication as to where and to what extent the thyroid cartilage needs to be deformed to obtain a maximum improvement in voice. Other preoperative tests include voice recording tomography and measurement of air flow rate during phonation. Laryngoscopic photographs are also taken when necessary. In cases of slight hoarseness with the vocal cord fixed in a paramedian position, thyroplasty is not the surgery of choice.

The other factors to be taken into consideration in deciding the indication of thyroplasty are 1) laryngeal findings, 2) type of the proceeding surgery, and 3) age. A scar or granulation on the vocal cord is associated with poor prognosis (Case 3). The thyroid cartilage may have sustained little or much damage after surgical intervention on the thyroid gland (Case 6). Surgery on the thyroid cartilage of the aged is generally difficult to perform due to advanced calcification and may require use of an oscillating saw (Case 11).

## SURGICAL PROCEDURES

The surgery can be performed on both in and out patients. Two of our patients were ambulatory while the other six were hospitalized for 2–3 days. The surgery is performed under local anesthesia with 1% Xylocaine. The patient is in the supine position with the head extended. Incision is made horizontally on the anterior neck at the level of the midpoint between the thyroid prominence and lower margin of the thyroid cartilage. The thyroid cartilage is well exposed by separation of the bilateral sternohyoid muscles and additional Xylocaine is injected into the thyroid perichondrium.

The types of incisions in the thyroid alae vary with the cases. The most typical incision now being used is a rectangular one 10 mm×3 mm, the upper margin of which should be at the level of the vocal cord (Fig 1). Prior to incision, the rectangular window is marked with pyocutanine so that the upper horizontal line can be located at the midpoint between the thyroid notch and lower margin of the thyroid cartilage and can be extended further back parallel to the lower margin of the thyroid alae. If the fragment to be depressed is located too high, it may cause bulging of the false vocal cord or Morgagni's ventricle, although we have never experienced such an occurrence. An anatomical study on the human cadaveric larynx indicated that the anterior commissure of the vocal cords (upper surface) is positioned almost at the level of the midpoint between the thyroid notch and lower margin of the thyroid cartilage or slightly higher than that level. Details of the measures will be given elsewhere. Incision is usually done using a No. 11 BP blade but may require an oscillating saw (narrowest gingko-shaped blade) in case of ossified cartilage (Case 11). Too deep incision and elevation beyond the inner perichondrium should be carefully avoided in order to minimize swelling or edema of the vocal cord. After gentle and careful marginal separation of the rectangular fragment with a rather fine raspatorium, the fragment is de-

pressed inward to varied depths to determine at degree of depression is optimal for voice reduction

During this procedure of adjusting depression to the best obtainable voice, the extended cord should be repositioned straight to avoid judgement due to the abnormal head position. If only slight inner displacement of the fragment is optimal for voice production, the pressed rectangular fragment is shifted 1 cm downward (caudal direction) behind the remaining frame of the thyroid alae. If further displacement is required for better voice production a wedge should be inserted between the rectangular fragment and the remaining frame of the thyroid. Usually a wedge taken from the upper margin of the opposite thyroid ala and may be pared to an adequate thickness. The wedge is usually fixed with the thyroid ala by one or two 4/0 nylon threads through sutures. Varieties of incisions such as two parallel incisions have also been described, as shown in Figs 1-6, 8, 9. At this stage of clinical experience, no conclusion can be drawn as to which is the best incision to be used but the rectangular incision with or without a wedge appears most effective with minimal intervention. The optimal type incision still remains as a future problem for study.

In cases where the paralysed cord is situated higher than the intact one cricothyroid approximation should be tested to determine whether there is further improvement in the voice. It is usually done by pulling a 3-0 nylon suture between the thyroid and cricoid cartilages. If voice improvement is obtained by the tentative pull, the thyroid and cricoid cartilages should be fixed in approximation with one or two 3-0 nylon mattress sutures. For prevention of perichondritis antibiotic powder is usually applied to the field before closure of the wound. The bilateral sternohyoid is sutured together to cover the cartilage. For cosmetic reasons closure of the skin incision should be done most carefully. Slight oedema of the involved vocal cord may last for

several post-operative days but never to the extent of causing stridor or dyspnea. In one case (No 11) dark red discoloration of the false vocal cord suggestive of submucous hematoma was noted, on the side from where the wedge had been taken. This reddishness disappeared in 2 weeks.

## RESULTS

Voice improvement was obtained in all operated patients, although the result in case of traffic trauma was unsatisfactory. Cases of thyroplasty I so far performed are too few to assess the results statistically. Therefore, each case will be described chronologically. The results were evaluated through auditory evaluation based on semantic differential methods, sonagram, computer analysis of pitch perturbation (mean of the cycle-by-cycle variation of the fundamental frequency expressed in semi tone = mean  $\Delta f$  in semi tone) (Table 1), and measurement of air flow during phonation.

Case No 2 S M, a male aged 27, with left vocal cord paralysis, which occurred after surgical removal of a jugular glioma tumour on the left side of the neck 1 year and 9 months

Table 1 Pitch perturbation in semi tone

Case No	Sex	Before surgery	After surgery
2	m	Unmeasurable	0.31
5	m	1.30	0.18
6	f	0.56	0.18
9	m	1.43	0.17
11	m	1.20	0.28
4	f	0.26	0.17
8	f	0.41	0.20

Pitch perturbation was expressed as the mean of the cycle-by-cycle variation of the fundamental frequency in semi tone

$$\overline{\Delta f} = \frac{\sum |F_i - F_{i-1}|}{N-1}$$

where  $F$  is an instantaneous frequency in semi tone and  $N$  is a number of measured waves. The mean value range of 95% confidence limit for the normal 0.16 and 0.08-0.23 for male and 0.19 and 0.11 female respectively.

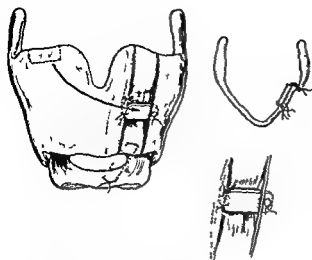


Fig 2 Thyroplasty type I and IV for case No. 2

previous to the surgery. The surgical record indicated that the vagal nerve had been sectioned at the time of surgery. His voice was extremely breathy and weak. Indirect laryngoscopy revealed the left vocal cord fixed almost in a lateral position. The fixed cord was found on radiotomographic examination to be positioned much higher than the intact cord.

Thyroplasty I and IV was performed as illustrated in Fig 2. Two parallel vertical incisions were made on the left ala and the midportion of the thyroid ala was depressed inwards. The improvement of voice was insufficient. A cartilage segment was removed from the upper margin of the opposite ala and used as a wedge to reinforce the compression effect particularly at the posterior part of the vocal cord. Since combined paralysis of the recurrent and superior laryngeal nerves was evident in this case, cricothyroid approximation was added to the above procedure, resulting in further improvement of voice. Although the postoperative improvement of voice was remarkable, the voice was still slightly hoarse and a minute difference in level between the two vocal cords was still evident on tomogram.

**Case No. 3** K. Y., a male aged 21, left vocal cord paralysis after traffic trauma to the neck. The patient had been severely hoarse for 7 years after the accident. Indirect laryngoscopy



Fig 3 Thyroplasty type I for case No. 3. The posterior one third portion of the thyroid cartilage was slipped in beneath the middle one third to increase compression effect at the posterior glottis. The lateral portion, however, was not markedly displaced medially, presumably because the cricoid cartilage blocked medial displacement of the lateral one third thyroid

revealed the left vocal cord fixed in an intermediate position and bilateral scar formation particularly in the subglottic region. On phonation, a triangular chink was noted at the posterior glottis. Manual inward depression of the superior horn of the thyroid cartilage produced some improvement of voice. Thyroplasty I was performed as illustrated in Fig 3.

First, a vertical incision was made on the left thyroid ala at anterior-middle third and the lateral part was slipped in beneath the medial part. The voice improvement was not remarkable. On the basis of the clinical finding that the glottal chink was wide posteriorly, another vertical incision was made and the posterior one-third portion was slipped in beneath the middle one third to increase the compression effect at the posterior glottis. However, the lateral portion was not markedly displaced medially, presumably because the cricoid cartilage blocked the medial displacement of the lateral one third thyroid ala. Postoperative improvement of voice was not to the degree expected before surgery, though the glottal chink during phonation was much reduced on laryngoscopy. It was assumed that the scar tissue on the vocal cord may be greatly responsible for the rather unsatisfactory results.

**Case No. 5** T. T., a male aged 36, left vocal

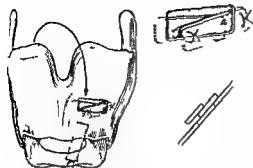


Fig 4 Thyroplasty type I and IV for case No 5. A wedge of cartilage was inserted beneath the thyroid ala and fixed with two 4-0 nylon sutures.

and paralysis attributed to pulmonary tuberculosis with a 6 month history of hoarseness. The left vocal cord was fixed in an intermediate position and positioned higher than the healthy one on tomogram. Manual compression of the thyroid alae inwards and cricothyroid approximation resulted in an almost normal voice.

The typical rectangular incision was made in the left thyroid ala as shown in Fig 4. A cartilage fragment was taken from the opposite side, pared off to a half thickness and fixed as a wedge inside the rectangular window. Cricothyroid approximation further improved the voice. The thyroid cartilage was partly calcified but not to the extent to make incision with the scalpel difficult. The voice after surgery was almost normal as shown in Table I.

**Case No 6 K O** a female aged 48 left vocal cord paralysis after surgery on the thyroid gland. Her voice had been hoarse and weak postoperatively. Exposure and vertical incision on the thyroid cartilage as shown in Fig 5 revealed fragility particularly at the median and lower part of the thyroid cartilage which was seemingly ascribed to electrocoagulation during the preceding surgery. The lateral fragment was slipped in beneath the median portion as was done in cases 2 and 3 but due to the fragile nature of the median portion of the thyroid cartilage the inward compression effect was insufficient. To enhance inward compression a cartilage frag-

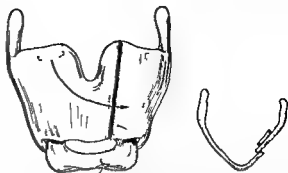


Fig 5 A typical thyroplasty type I and IV for case No 6. The cartilage fragment was inserted beneath the thyroid ala. Due to the fragile quality of the thyroid ala, typical thyroplasty type I was impossible.

ment taken from the opposite side was inserted beneath the left thyroid ala at the level of the vocal cord (Fig 5). The lateral part of the left ala was again slipped in beneath the median part.

The left cricothyroid muscle was scarred and atrophic and did not contract on phonation in contrast to the right cricothyroid muscle. A 3-0 nylon suture was tied to shorten the cricothyroid distance. Again due to the fragility a strong pull was impossible. The voice after surgery was close to normal but on occasion slightly fluttering in quality especially when the head was inclined toward the right. The difference in level between the two cords was not completely corrected.

**Case No 9 K T** a male aged 27 right vocal cord paralysis of unknown etiology (in intermediate position). The manipulation test indicated good prognosis after surgery. Typical rectangular form thyroplasty I with a wedge was performed (Fig 6). Cricothyroid approximation was not done in this case because the tentative cricothyroid approximation resulted in a rather cracking voice. The voice after surgery was quite satisfactory as shown in Fig 7.

**Case No 11 S N** a male aged 64 left vocal cord paralysis of unknown etiology of 1 year 7 months duration. The vocal cord was fixed in an intermediate position. The difference from the intact cord

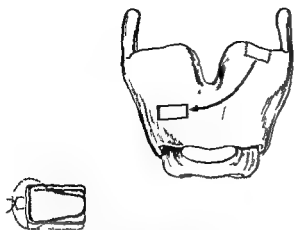


Fig. 6 Thyroplasty type I for case No. 9. A wedge is used to increase the lateral compression particularly at the posterior portion of the vocal cord.

tion test indicated good prognosis. The rectangular incision was made in the thyroid ala, which was so calcified that an oscillating saw was required for cutting.

The narrowest ginkgo shaped blade, used to cut 80% through the full thickness. A No. 11 blade was used to complete the incision. An almost normal voice was obtained when the rectangular fragment was depressed deep inside. A wedge was taken as usual; a half thickness wedge was inserted perpendicular to the rectangular window (Fig. 1). The procedure of inserting the wedge was very difficult and required considerable care. Postoperatively, the right false vocal cord was reddish and swollen for a week, probably due to slight hematoma after removal of the cartilage fragment. As the swelling subsided, the voice improved almost to a normal quality.

**Case No. 4.** A 1, a female aged 30, vocal cord atrophy. Three years previously she had used oral contraceptive pills for a few months. Her voice had been hoarse since then. Indirect laryngoscopy revealed a marked oval glottis.

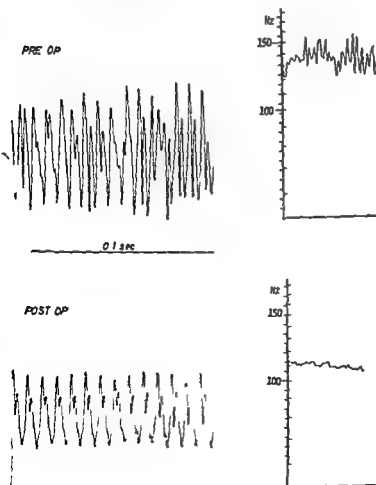


Fig. 7 The acoustic wave of voice (left) and pitch perturbation (right) in case No. 4 before and after surgery. For pitch perturbation graph, the ordinate indicates frequency scale in semi tone while the abscissa indicates the consecutive number of sound waves. The pitch perturbation was markedly reduced after surgery.

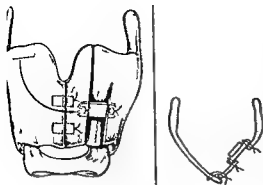


Fig 8 Thyroplasty type I for case No 4

nk during phonation despite apparently normal movement of the bilateral vocal cords. Neither scar nor sulcus was noted on the vocal folds. Voice training did not result in improvement in the glottal condition but the likelihood of the glottal chink changed day by day. Based on the finding that her voice was greatly improved by manual compression of the thyroid cartilage she was hospitalized for thyroplasty type I. The thyroid cartilage was incised vertically in the median line and the ala was shifted inward and fixed with 4-0 nylon sutures (Fig 8). The voice was much improved during surgery.

The laryngoscopic findings on the next day were rather discouraging with a noticeable chink still remaining on phonation particularly at the posterior glottis. Her voice resolved after surgery. The possible factors responsible for the change of voice from the time of surgery may be: 1. extended neck at the time of surgery; 2. postoperative laryngeal edema which may suppress a normal contraction of the laryngeal muscles during phonation; 3. postoperative edema.

After being followed up for 3 weeks she was reoperated to reduce the posterior glottal chink during phonation. Two vertical incisions were made on the left thyroid ala as shown in Fig 8. The middle cartilagenous portion with a pedicle upward was depressed inward with a wedge taken from the opposite side ala. The edge was fixed with two 4-0 nylon sutures. After this second surgery her speaking voice

was much improved but the glottal chink noted on laryngoscopy during phonation still remained though to a lesser degree. The degree of imperfect closure of the glottis fluctuated greatly day by day.

**Case No 8 K T**, a female aged 18, vocal cord atrophy. She had a complaint of laryngeal pain on phonation, slight hoarseness, weak and too-high pitched voice of 8 years duration. Voice therapy in an attempt to lower the pitch was ineffective. Linear chink during phonation was noted on indirect laryngoscopy. Slight compression of the thyroid ala improved the pitch and quality of voice. Typical thyroplasty type I without a wedge was performed (Fig 9). According to her statement, she became completely free from pain on phonation one week after surgery. Her voice was very much improved in quality but still sounded slightly asthenic.

## DISCUSSION

Our previous report covered the various surgical methods developed by many surgeons for correcting the fixed vocal cord position. Among them, teflon or silicon injection or cartilage implantation are presently the most popular. Intrachordal injection of plastic material is certainly a good and simple method to bring the vocal cord margin toward the midline. However, Bernstein & Holt (1967) pointed out possible disadvantages of the method such as neoplastic response or

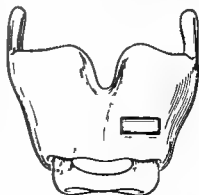


Fig 9 Thyroplasty type I for case No 8



eventual rejection by the tissue of the synthetic materials, migration or absorption of the implanted tissue, local stiffening of the vocal cord due to injection etc. We also obtained excellent results in voice production by intrachordal injection of elastic silicon in many patients with vocal cord paralysis. In a few cases, however, the results were unsatisfactory especially when too much elastic silicon was injected near the margin of the vocal cord in a second or third injection, resulting in rather stiff vocal cord. In other words, one of the disadvantages of the injection method is that the volume and site of injection cannot be altered according to the voice, since the injected materials cannot be removed.

The advantages of our methods of thyroplasty are: 1. Adjustment of the degree of lateral compression is possible during surgery in accordance to the voice obtainable. 2. Intervention inside the inner perichondrium of the thyroid cartilage is minimal. Although our experiences with thyroplasty are not so extensive, we have encountered problems, as described for each case. They were: 1. extended head position, 2. previous scar on the vocal cord or the thyroid cartilage, 3. calcified cartilage, 4. postoperative edema, 5. functional aspect of vocal cord atrophy.

We have not yet concluded which type of incision on the thyroid ala is best. After the experience with two vertical parallel incisions, we are inclined to prefer the rectangular type as it seems sufficiently effective and involves less surgical trauma. This type of incision is very similar to that used by Payr (1915), but differs in so far as ours has no pedicle so that the fragment can shift quite easily toward the midline. The site and size of cartilage incision should be based upon anatomical data of the human larynx. Our data regarding various measurements of the human larynx which should be referred to when considering thyroplasty, will be reported elsewhere.

In each case we examined whether or not the cricothyroid approximation by suture im-

proves the voice after thyroplasty type I. As is well known, there is the possibility of combined recurrent and superior laryngeal nerve paralysis when the vocal cord is fixed in an intermediate position, according to Wagner-Grossman. Our preliminary experimental study on unilateral paralysis of the cricothyroid muscle demonstrated that thyroplasty alone does not cause hoarseness but rather there is a lag in the vibratory phase between the two cords. If it is accompanied by imperfect closure of the glottis the results appear rather complex. Such is now under study utilizing an extirpated larynx and a high speed motion camera. The effect of cricothyroid approximation on the level of the vocal cord remains to be further investigated.

In our experiments of sectioning the recurrent laryngeal nerve, additional sectioning of the ipsilateral external branch of the superior laryngeal nerve resulted without exception in relative elevation of the immobilized vocal cord by 1 mm. and cricothyroid approximation by suture corrected that level difference of the two vocal cords. On the basis of this experimental finding we make a tentative cricothyroid approximation a routine procedure during surgery in cases where the vocal cords are positioned at different levels. Factors other than the cricothyroid muscle apparently affect the level of the vocal cord: the more lateral the fixed position the higher is the one paralysed cord.

Manipulation of the laryngeal cartilage before surgery not only indicates the post-operative prognosis but also helps relieve the patient's anxiety as to what his voice would be like after surgery. Our clinical experiences with thyroplasty I indicate its usefulness for dysphonia due to vocal cord paralysis or atrophy. As experience accumulates modification of techniques will necessarily follow and these shall be reported in following papers.

## ZUSAMMENFASSUNG

Auf Grund der experimentellen Resultate der Thyroplastik wurde Thyroplastik Typ I die med. ala Verschiebung

der Stimmbänder) bei acht Patienten mit Dysphonie  
auch mit Stimmbandlähmung zwei mit Stimmbandatro-  
phie) ausgeführt. Die chirurgischen Eingriffe wurden bei  
einigen entweder ambulant oder stationär aufgenommenen  
Patienten unter lokaler Anästhesie durchgeführt. Der  
sublinguale Einschnitt des Schildknorpels wurde im  
Zentrum in der Höhe der Stimmbänder vorgenommen  
und das Bruchstück wurde nach innen gedrückt. Das von  
der anderen Seite herausgenommene Knorpelstück  
wurde wenn notwendig als Keil benutzt und die Wirkung  
der lateralen Expansion auf die Stimmbänder zu erhöhen.  
Nach dem Eingriff war die Stimme im allgemeinen ge-  
wöhnlich. Ein Fall mit Stimmbandlähmung nach einem  
Schlaganfall ausgenommen. Es gab insgesamt keine  
Nebenwirkungen (Stidor oder Dysphagie). Weil der chirur-  
gische Eingriff im Inneren des Schildknorpels minimal ist  
und die feine und sichere Regulierung des Niederdrucks  
operativ ohne weiteres durchgeführt werden

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## IMMUNOLOGICAL FUNCTION OF HUMAN TONSIL

### *Surface Topology of Human Tonsil Lymphocytes using the Scanning Electron Microscope (SEM)*

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**Abstract** The surface topology of the lymphocytes in human tonsils was performed by means of the scanning electron microscope (SEM). It was revealed that human tonsils consist of both 40.7% villous and 26.6% smooth surface lymphocytes. In general, the percentage results agree with those of E and EAC binding lymphocytes of human tonsils as previously reported. Our serial experiments using immunological techniques proved the existence of two types of lymphocytes (T and B cell) having immunological roles.

In our recent investigation of the immunological roles of human tonsil lymphocytes, it was proved that the lymphocytes of tonsils can be differentiated into two subpopulations—bone marrow derived (B) and thymus-dependent (T) cells—which have characteristic membrane markers rosetting with sheep red cell (Tabata et al., 1974). Further investigation, using an immunofluorescent technique revealed that human tonsils contain a certain type of lymphocyte (B cell) with immunoglobulin on their surfaces (Tabata et al., 1974). The present study is an attempt to characterize the surface topology of human tonsil lymphocytes by using the scanning electron microscope.

## MATERIALS AND METHODS

Experimental materials were obtained from patients with hypertrophied tonsils due to the recurrence of chronic inflammation. The patients were otherwise free of systemic disease or complicating factors.

### *Lymphocyte preparation*

The preparation of lymphocytic suspensions from human hypertrophied tonsils using Angi-Conray Ficoll was performed according to our previous report (Tabata et al., 1973). The cells of approximate  $10^7$  were put on a silver membrane with 0.8  $\mu$ m porosity (Flotrack Spring House, Pa.) by aspiration. The silver membranes with the harvested cells were fixed at least half an hour in pH 7.3 4% glutaraldehyde and rinsed twice with pH 7.3 phosphate buffer. Subsequently, postfixation was carried out in 1% osmium tetroxide for 1 hour and rinsed again in the buffer. Section was performed in a graded series of alcohol for 5 min each. The silver membrane with fixed and dehydrated monolayered cells was prepared for critical point drying in carbon dioxide. Critical point drying of the specimen was performed as described by Pollak et al. (1973). The portions of the silver membrane were then coated with a thin layer of carbon and gold on a rotating stage.

### *Observation of specimens*

ISM-35 scanning electron microscope was operated at an accelerating voltage of 30 kV.

## RESULTS

The clinical conditions and the percentages of cell types of each sample were summarized in Table 1. An actual counting of 150 cells from

Table 1. A summarized result of the percentages of smooth and villous cells

Sex	Age	Clinical diagnosis	Smooth cells (T) (%)	Villous cells (B) (%)	Indistinguishable cells (%)
Female	13	Hypertrophic tonsil	31.4	33.3	35.3
Male	34	Hypertrophic tonsil	24.3	47.2	28.5
Male	10	Hypertrophic tonsil	31.1	41.6	34.3
Mean			26.6	40.7	32.7

TEM micrographs was performed from each sample. Fig. 1 is a general view of the monolayered tonsil lymphocytes under SEM. It can be seen from the Table that from 33.47–2% of the tonsil lymphocytes had irregular surface architecture demonstrating protrusions (microvilli) with various lengths (Fig. 2). Fairly smooth cells without protrusions on the surface on the other hand were also observed (Fig. 3). The percentage of these smooth cells ranged from 24.1–31.4%. It is a fact that there are indistinguishable cells from the identical two types of cells having either long or shortened protrusions on the cell surfaces (Fig. 4).

## DISCUSSION

In 1974 Tabata et al. reported that subpopulations of the lymphocytes from human tonsils were capable of rosette formation with SRC and SRC sensitized with anti SRC antibody complement. It was accepted that human tonsils may well supply two different kinds of lymphocytes that is bone marrow derived (B) and thymus dependent (T) cells and which produce antibody with their collaboration.

Recently scanning electron microscopy has been used to reveal the surface architecture of lymphocytes.

In 1973 Polliak et al. announced that distinguishing between normal B and T lymphocytes

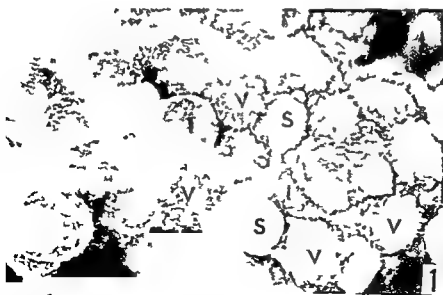


Fig. 1. A low magnification of monolayered tonsil lymphocytes  $\times 3000$ . V Villous cells (B cell equivalent). S Smooth cells (T cell equivalent). I Indistinguishable cells.



Fig 2 Typical villous cells  $\times 10000$

of human peripheral blood (PBL) was relatively easy, in most instances on the basis of their surface architecture under SEM. It was confirmed that 20% of the lymphocytes of PBL had villous surfaces identical with B cells, while the majority of T cells (80%) were relatively smooth and smaller in size.

Our present study on human tonsil lymphocytes revealed that the proportion of the lymphocytes with villous surfaces reached 40.7% on the average. Contrary to the percentage of villous lymphocytes (26.6%) was the average of the smooth lymphocytes. It is assumed that a large proportion of T cells from

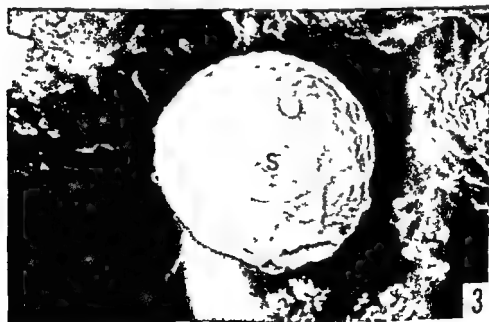


Fig 3 Smooth cells without distinct villi  $\times 10000$



Fig 4 Indistinguishable cell having fewer and shorter microvilli. Adjacent villous cell  $\times 10000$

report by Polliak and his co-workers are attributable to involvement of the relative proportions of the two cell types. In our experiment, however, these cells were separated into indistinguishable cell groups because of lack of evidence of distinction. It is of interest, however, that the proportion of villous and smooth cells by SEM topology corresponds with that of E (22.6%) and EAC (7.7%) binding lymphocytes of human tonsils previously reported by Tabata et al (1974). It is well known, furthermore, that B cells in humans and mice have easily demonstrable surface immunoglobulins on their surface which interact with antigen. Recently, a membrane immunofluorescence technique for detection of B lymphocytes in human tonsils (Tabata et al 1974) proved that on the average approximately

30% (20–35.4%) of the cells had polyvalent surface immunoglobulins on their surface. For easy understanding, the typical cells having surface immunoglobulins are shown in Fig 5. Similar results of the presence of approximately 30% surface immunoglobulin bearing cells in human spleen were reported by Grey et al (1971).

The slight discrepancy in the percentages between surface immunoglobulin bearing cells and villous cells in human tonsil lymphocytes will be resolved when attention is given to discovering the real characteristics of indistinguishable lymphocytes under SEM.

#### ACKNOWLEDGEMENT

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Fig 5 Surface immunoglobulin bearing lymphocytes from human tonsils by membrane immunofluorescence  $\times 400$ . A ring B patchy B capping

## ZUSAMMENFASSUNG

Eine Oberflächen Topologie der in den menschlichen Tonsillen befindlichen Lymphozyten ist mit dem Raster-elektronmikroskop durchgeführt worden. Es wurde deutlich, dass die menschlichen Tonsillen aus 40-7% zottigen und 26-6% glatten Lymphozyten bestehen. Im allgemeinen stimmen diese Prozentsätze mit denen der E- und EAC bindenden Lymphozyten der menschlichen Tonsillen überein. Es scheint mir aber, dass noch ununterscheidbare Zellen existieren, wenn auch eine experimentelle Bestätigung fehlt. Eine Reihe von Experimenten mit immunologischer Technik bestätigt nun die Existenz der 2 Typen von Lymphozyten (T- und B-Zelle), die eine immunologische Rolle spielen.

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